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Release Mechanisms of 5-HT from the Gastrointestiual Tract in Rats

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Introduction

Since ERSPAMER and ASERO (1952) it is established that serotonin (5-HT) is produced by the enterochromaffin (EC) cell, one of the typical elements of the gastro-entero-pancreatic (GEP) endocrine system (cf. FUJITA, 1973). Distribution of 5-HT in human gastrointestinal tract has been demonstrated by fluorescence histochemical method of FALCK and HILLARP (TOBE, TANAKA and FUJIWARA, 1966).

Release of 5-HT from the EC cells by local luminal stimuli has been studied by our research group. By combined bioassay and fluorescence histochemical method, we revealed that 5-HT in the human and canine duodenal EC cells are markedly decreased after luminal administration of hypertonic glucose. On the basis of this and other findings we suggested that human dumping syndrome after gastrectomy might be caused by 5-HT released from the gut stimulated by hypertonic foodstuffs (TOBE, KIMURA and FUJIWARA, 1967a). Release of granule contents from the canine EC cells stimulated by hypertonic glucose has later been confirmed by electron microscopy by KOBAYASHI and FUJITA (1973).

In the present communication, another mechanism of 5-HT release from the gastrointestinal EC cells will be dealt with——that is the vagal release of 5-HT. A part of this study has been reported elsewhere (TOBE, et al., 1974).

This paper describes an experiment designed to clarify the mechanism of release of 5-HT from the gastrointestinal tract in rats.

Materials and methods

The experimental animals were 300 male Wistar rats weighing 200-250 grams. As a rule, five rats per group were used, but the number was increased when necessary. The 5-HT level in portal vein blood and in the gastroduodenum was determined at set intervals after truncal vagotomy, after electric vagal stimulation, and after the administration of various autonomic drugs. The results were averaged and the standard errors and significant

Key words : 5-HT, Enterochromaffin cell, Fluorescence histochemical method, Vagal release, Vagotomy.
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differences were determined by "Student's t-test". Fluorescent histochemical studies were done in each group. The autonomic drugs were used hexamethonium, neostigmin, atropine and 6-hydroxydopamine (6-OH-DA).

1) Method of truncal vagotomy : Rats were fasted 24 hours (water was permitted) and anesthetized by intra-peritoneal injection of 30 mg/kg of nembutal. After laparotomy by the method of SNOWDON (1970), both the anterior and posterior roots of the vagus were severed subdiaphragmatically. To insure the effect of the vagotomy, the esophagus was peeled and separated to show the mucosa, and the incision was closed. Fifteen rats each in the lst, 2nd and 3rd weeks after truncal vagotomy were fasted 48 hours (water ad lib), and after their stomachs had been completely evacuated, they were decapitated, and 5-HT level chemical assays were done of the stomach corpus and antrum and the duodenum. Any rats with food in the stomach were excluded. For the fluorescent histochemical analysis, the antrum, which has a large 5-HT content, was used. The 15 control rats were laparotomized but vagotomy was not performed, and after 48 hours of fasting, they were analyzed with the same procedures as the 15 vagotomized rats. Ten rats were used for 5-HT chemical assay of the tissues, and five rats were used for fluorescent histochemical analysis.

2) Determination of 5-HT in portal vein blood : For the collection of portal vein blood, a No. 23 needle was attached to a disposable 1 ml syringe containing 0.2 ml of sodium citrate. This needle was inserted into the portal vein, and 0.8 ml of blood was drawn, shaken well, placed in a centrifuge tube containing 5 ml of 0.4 N $HClO_4$, cooled over ice and chemically assayed for 5-HT.

3) Method of electric vagal stimulation : Rats were fasted for 24 hours (water ad lib) and anesthetized with an intra-peritoneal injection of 30 mg/kg of nembutal. An upper-abdominal mid-line incision was made, and the anterior and posterior roots of the vagus were peeled and separated subdiaphragmatically. After ligation with a No. 3 silk thread, the branches were severed proximally, and the distal ends were bent down and placed on a bipolar platinum electrode connected to a Sanei stimulator (ES 103 Electrical Stimulator) delivering monophasic square wave stimuli with a duration of 5 msec, 10 c/s (frequency) and an intensity of 4 V. The abdominal vagus nerves were used so that the heart and lung would not be affected. To prevent drying of the vagal nerves attached to the platinum electrodes, liquid paraffin was poured over them. At set intervals, portal vein blood and gastroduodenal tissue were collected and chemically assayed for 5-HT.

4) Quantitative assay of 5-HT : The method of BOGDANSKI et al. (1956) was used.

(i) Collecting of experimental tissue and making of homogentate : Decapitation, or laparotomy under nembutal anesthesia, extirpation of the gastroduodenum, division, and weighing were done as fast as possible (about 5 minutes). In the vagotomized rats, adhesions were sometimes strong and a longer time was required (approximately 10-15 minutes), so the operations were usually performed over crushed ice. The stomach corpus, antrum and duogenum were weighed and homogenized with 5 ml of cooled 0.4 N $HClO_4$. Whole blood was used. Later, 2-3 drops of 0.5% ascorbic acid were added to stabilize the 5-HT.

(ii) Extraction of 5-HT : For the extraction of 5-HT, three groups of three sets of 50 ml Pyrex culture tubes with screw caps, were used, each set consisting of determination test+standard (4-6 tubes) and reagent blank (2 tubes). The test chemicals were :

(1) 5 g NaCl
15 ml butanol (NaCl sat. H_2O washed)
10 ml borate-carbonate buffer (pH 10.7)
(2) 10 ml borate buffer (pH 10.4)
(3) 20 ml heptane (acid washed)
3 ml 0.1 N HCl

The homogenate was added to glass tube (1) containing the test-chmicals (1), shaken for 15 minutes, then centrifuged at 2,000 rpm. The butanol phase and the water phase separated into the upper and lower parts of the tube. Between these two layers was the homogenate phase of the tissue. At this time, the pH was 10.0-10.4 and the basic 5-HT moved to the butanol phase and the neutral amino acid, 5-HT and the acid 5-HIAA were removed. Then, 12 ml of the butanol phase was aspirated by pipette and decanted into glass tube (2) containing borate buffer, shaken for 15 minutes, then centrifuged for five minutes. The upper layer was butanol phase containing 5-HT. Then, 10 ml of the butanol phase was aspirated by pipette and decanted into glass tube (3) containing heptane and 0.1 N HCl, shaken for 15 minutes, then centrifuged for five minutes. The upper layer was a mixture of heptane and butanol, the lower layer was 0.1 N HCl containing 5-HT. The organic solvent layer was removed by aspiration, and 2 ml of the HCl layer was decanted into a test tube for determination of fluorescence.

(iii) Determination of 5-HT fluorescence : To 2 ml of the 0.1 N HCl layer, 0.6 ml of 12 N HCl was added to make the final density 3 N HCl, and a Hitachi fluorescence spectrophotometer or an Aminco-Bowman Co. fluorescence spectrophotometer was used to determine the activation wavelength of 300 mμ and the fluorescence wavelength of 540 mμ.

5) Fluorescent histochemical analysis of 5-HT by FALCK-HILLARP's method (1962).

(i) Collection of histological samples.

After decapitation of laparotomy under nembutal anesthesia by intraperitoneal injection, histological samples were taken from the gastroduodenum and frozen. The samples, less than 0.5 cm in diameter, were fixed to glass slides.

(ii) Freeze-drying method.

To freeze the samples the glass slides were placed in isopentane, which when cooled with liquid nitrogen reaches a super-low temperature of $-165°C$, and frozen instantaneously. At this time, if the samples were large, ice-crystals formed and caused changes in the intra-cellular structure. After freezing, the sample was peeled from the glass slide and placed in a gauze bag. For vacuum drying this gauze bag was placed in an appropriately sized beaker, which in turn was placed in a flask containing phosphorus pentoxide. This experimental flask was connected to a freeze drying apparatus, and the sample was vacuum dried at $-35°C$ for approximately one week. After a week, the compressor was stopped

and the sample returned to room temperature while vacuum drying was continued.

(iii) Condensation reaction by formaldehyde gas.

The dry sample and 5 g/l of paraformaldedhyde were placed in an airtight glasscontainer and heated to 80°C in an incubator for one hour. Since the reaction requires a certain humidity in the formaldehyde vapour, paraformaldehyde and sulfuric acid at a specific gravity of 1.2-1.3, depending on the season, were placed in an airtight glasscontainer for about a week.

(iV) Embedding and microtomy.

The sample which had been treated with formaldehyde gas was immediately immersed in paraffin wax (melting point 56-58°C) and connected to a vacuum pump and infiltrated at 60°C vacuum for one hour. Next, the sample was embedded in paraffin and made into a block and, after being attached to a piece of wood, it was sliced at 4-8 μ with a microtome. The sliced sample was set on a non-fluorescent glass slide and heated on a hot plate to 60°C and stretched. The paraffin was then removed by adding 1-2 drops of xylene. The sample was then enveloped in a 1 : 1 mixture of xylene and Entellan (Merck) and examined microscopically.

(V) Microscope examination and photography

For microscope examination either a Zeiss or a Leitz fluorescence microscope was used. For the former a high pressure mercury lamp (Osram HBO 200) was used as the light source, and a primary filter BG 12 and secondary filter zeiss "50" were used. Photography was done with Kodak Ektachrome film ASA 160 with an exposure time of 1-3 minutes or Kodak TriX ASA 400 with an exposure time of 20 seconds to one minute.

6) Administration of various autonomic drugs.

(i) Hexamethonium 5 mg/kg was injected intra-peritoneally, and 5-HT was assayed chemically 30 minutes later.

(ii) Thirty minutes after the intra-peritoneal injection of 0.1 mg/kg of neostigmine 5-HT was assayed chemically.

(iii) 5-HT was assayed chemically 30 minutes after the intra-peritoneal injection of 0.01 mg/kg of atropine.

(iV) One week after the intra-peritoneal injection of 200 mg/kg of 6-OH-DA 5-HT was analyzed.

Results

1. Effect of Vagotomy on 5-HT Levels in Portal Vein Blood and in the Gastroduodenum

1) Postoperative weight curve

The weight of the control rats increased approximately 20 gms each week, but after truncal vagotomy rats lost weight for up to two weeks after the operation, then gained weight at about the same rate as the control rats (Fig. 1).

2) Pre- and post-vagotomy weight of stomach tissue accompanying body weight changes

The stomachs of the vagotomized rats were larger and heavier and the walls were

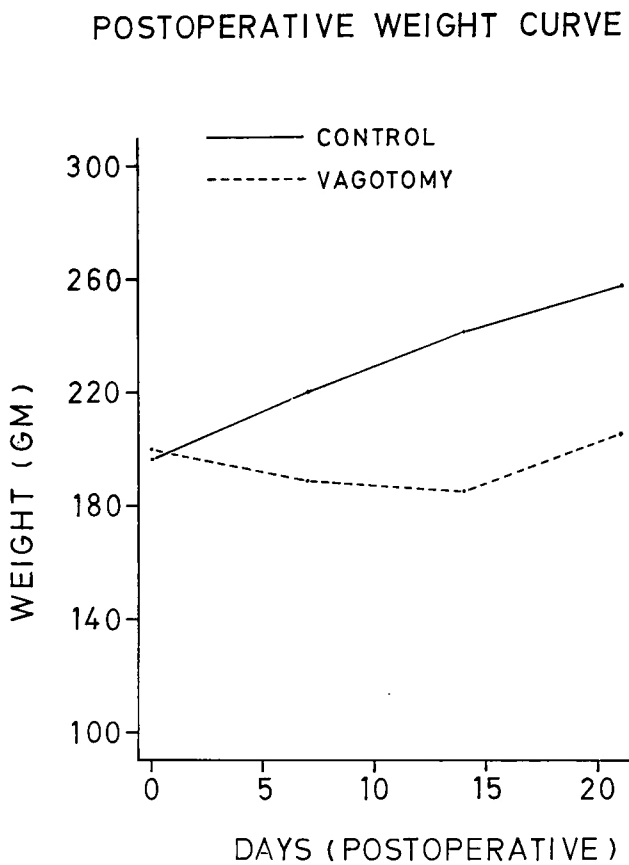

Fig. 1 The weight of the control rats increased approximately 20 gms each weeks, but after truncal vagotomy rats lost weight for up to two weeks after the operation, then gained weight at about the same rate as the control rats.

thicker than those of the control rats. One week after vatotomy, the antrum was proportionately the heaviest part of the stomach, two weeks after the vagotomy, the corpus and three weeks after vagotomy the rumen were heaviest (Table 1).

3) 5-HT levels in stomach and duodenum

Corpus : The 5-HT level in the corpus one week after vagotomy was 3.31 ± 0.28 $\mu g/g$, compared with 3.58 ± 0.34 $\mu g/g$ in the control rats (a slight but not significant decrease). Two weeks after vagotomy, the 5-HT level was 3.77 ± 0.40 $\mu g/g$; three weeks after vagotomy, it was 4.28 ± 0.48 $\mu g/g$, indicating a tendency to increase, but not a significant difference.

Antrum : The 5-HT level in the control rats was 6.56 ± 0.32 $\mu g/g$ and already at one week after vagotomy it was 7.61 ± 0.43 $\mu g/g$ indicating a tendency to increase. The 5-HT level two weeks after vagotomy was 7.90 ± 0.56 $\mu g/g$, a significant increase ($p < 0.05$). Three weeks after vagotomy, the level was 7.98 ± 0.41 $\mu g/g$.

Table 1. One week after vagotomy, the antrum was proportionately the heaviest part of the stomach, two weeks after the vagotomy, the corpus and three weeks after vagotomy the rumen were heaviest.

Gram of wet tissue per gram of body weight in the rat

Treatment	Whole stomach	Body	Antrum
None	0.39%	0.31%	0.07%
1 Week after Vagatomy	—	0.31%	0.08%
2 Weeks after Vagatomy	—	0.44%	0.11%
3 Weeks after Vagatomy	0.53%	0.35%	0.10%

Table 2. The 5-HT level in the antrum 2 weeks after vagotomy increased significantly ($P < 0.05$).
In the duodenum, 3 weeks after vagotomy, it was significant increase.

Serotonin Contents in the Rat Stomach and Duodenum ($\mu g/g$)

Treatment	Stomach		Duodenum
	Body	Antrum	
None	3.58±0.34 (10)	6.56±0.32 (10)	7.13±0.27 (10)
1 Week after Vagotomy	3.31±0.28 (10)	7.61±0.43 (10)	6.75±0.73 (10)
2 Weeks after Vagotomy	3.77±0.40 (10)	7.90±0.56* (10)	8.04±0.62 (10)
3 Weeks after Vagotomy	4.28±0.48 (10)	7.98±0.41* (10)	8.42±0.22* (10)

*studnt's t-test $p < 0.05$

Duodenum : The 5-HT level in the control rats was 7.13±0.27 $\mu g/g$. One week after vagotomy it was 6.75±0.73 $\mu g/g$ a slight but not significant decrease. Two weeks after vagotomy it was 8.04±0.62 $\mu g/g$, a slight but not significant increase. Three weeks after vagotomy, it was 8.42±0.22 $\mu g/g$, a significant increase ($p < 0.05$) (Table 2, Fig. 2 Bottom)

4) 5-HT level of portal vein blood

The 5-HT level of portal vein blood in the control rats was 0.86±0.02 $\mu g/ml$ and in the vagotomized rats it was 0.83±0.03 $\mu g/ml$, i. e. no difference (Fig. 2 Top).

5) Fluorescent histological findings

The antrum, where enterochromaffin (EC) cells have a high 5-HT content, was examined. In the mucosal layer of the antrum, yellow 5-HT fluorescence corresponding to the EC cells was seen, mainly from the central to the lower part of the mucosal layer. Yellow 5-HT fluorescence was seen in the mast cells of the submucosal and mucosal layers. Green catecholamine (CA) fluorescence was seen in the sympathetic nerves surrounding the blood vessels of the submucosal layer. CA fluorescent sympathetic nerve terminals were also seen in Auerbach's plexus and between the longitudinal and circular muscles (Fig. 3A). Strong magnification (400×) showed CA fluorescent sympathetic nerve terminals close to the EC cells (Fig. 3B).

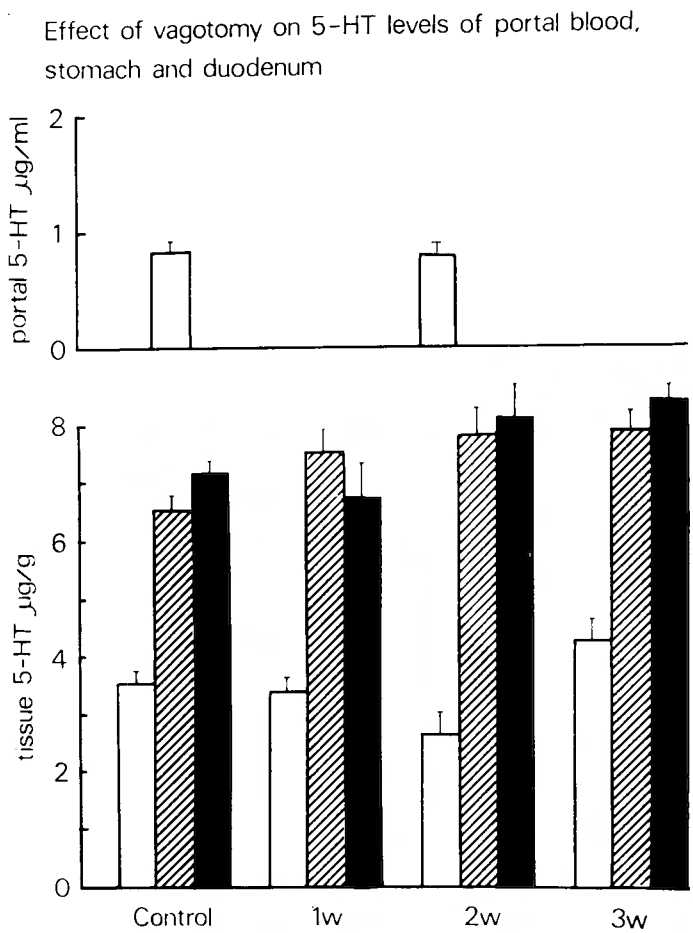

Fig. 2 **Top** The 5-HT level of portal vein blood in the control rats was 0.86±0.02 μg/ml and in the vagotomized rats it was 0.83±0.03 μg/ml, that is no difference.
Bottom Effect of vagotomy on the gastroduodenal 5-HT level. There was no significant change in the 5-HT level in the corpus after vagotomy. (white bars). The 5-HT level in the antrum (lined bars) showed significant increase 2 weeks after vagotomy. The 5-HT level in the duodenum (Black bars) showed significant increase 3 weeks after vagotomy.

One week after vagotomy : Almost no change was seen in CA fluorescence in the sympathetic nerve terminals or in 5-HT fluorescence of EC cells and mast cells.

Two weeks after vagotomy : The yellow 5-HT fluorescence of the mucosal layer was conspicuously increased, and the entire mucosa seemed brighter. On the other hand, the green CA fluorescence surrounding the blood vessels of the submucosal layer and within the muscle layer was not noticeably changed. There was also no change in the mast cells of the submucosal layer (Fig. 4A).

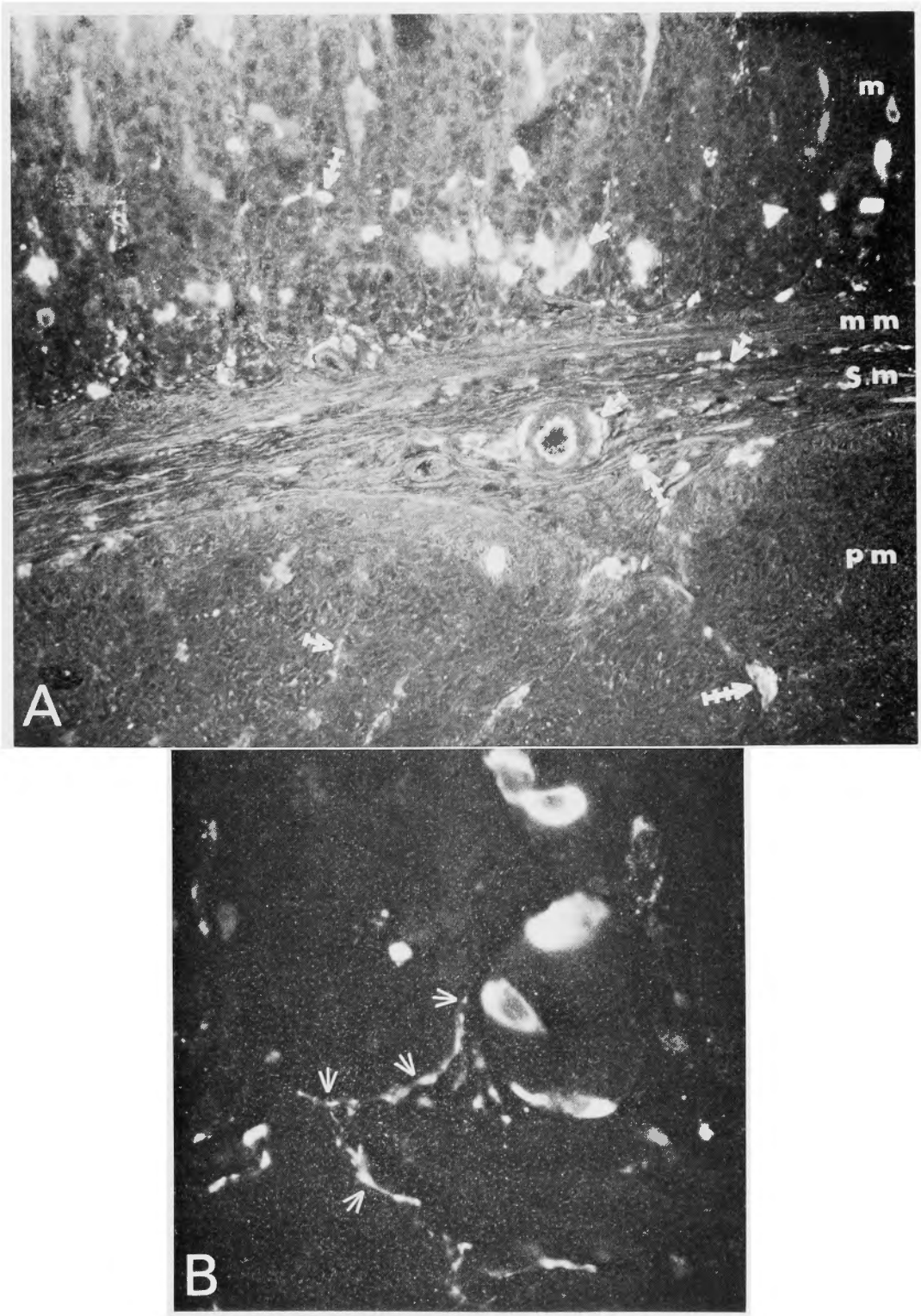
m
m m
S m
p m
A
B

Fig. 3A Fluorescence histochemical findings of antrum in rats. In the bottom 1/3 of the mucosa (m), 5-HT fluorescence corresponding to the EC cells (↑) was seen. Rather small oval 5-HT fluorescence corresponding to mast cells (⇑) was seen. Catecholamine fluorescence (↥) in the muscularis mucosae (mm), in the submucosal layer (sm), and in the proper muscular coat (pm) was seen. The fluorescence seen around the artery of the submucosal layer is also catecholamine fluorescence (↥). 5-HT fluorescence can be seen in the submucosal layer of mast cells. Between the longitudinal and circular muscles of the proper muscular coat the Auerbach's plexus (⇑) can be seen. (160×)

Fig. 3B Catecholamine fluorescent sympathetic nerve terminals (↑) was seen near the EC cells. (400×)

Three weeks after vagotomy : Yellow 5-HT fluorescence corresponding to the EC cells of the mucosal layer was clearly increased. There were no conspicuous changes in the green linear CA fluorescence in the vascular area of the submucosal layer, or in the muscularis mucosae (Fig. 4B).

Four weeks after vagotomy : There was a conspicuous increase in yellow 5-HT fluorescence corresponding to the EC cells of the mucosal layer. There were no noticeable changes in the green CA fluorescence surrounding the arteries of the submucosal layer or within the muscle layer.

2. Effect of Vagal Stimulation on 5-HT Levels in Portal Vein Blood and in the Gastroduodenum

1) 5-HT levels in portal vein blood

The 5-HT level in portal vein blood in the control rats was 0.86 ± 0.02 μg/ml. After five minutes of electric vagal stimulation (5 ms, 10 Hz, 4 V), the level was 1.15 ± 0.06 μg/ml ; after 15 minutes of electric vagal stimulation it was 1.61 ± 0.13 μg/ml and after 30 minutes, it was 1.08 ± 0.01 μg/ml. The 5-HT level in portal vein blood was highest after 15 minutes of electric vagal stimulation, or 86.5% above that in the control rats (Fig.5 top).

2) Gastroduodenal 5-HT levels

The 5-HT level in the corpus of the control rats was 3.58 ± 0.34 μg/g. After five minutes of vagal stimulation, it was 2.47 ± 0.19 μg/g ; after 15 minutes, it was 1.61 ± 0.21 μg/g ; after 30 minutes, it was 3.23 ± 0.17 μg/g. Under all conditions of stimulation, the 5-HT levels decreased conspicuously, but when the increase of 5-HT in portal vein blood was greatest after 15 minutes of stimulation, the 5-HT level in the corpus was the lowest, approximately 55% lower than in the control rats.

The 5-HT levels of the antrum and duodenum in control rats were, respectively, 6.56 ± 0.32 μg/g and 7.13 ± 0.27 μg/g, approximately twice the level in the corpus. The 5-HT levels in the antrum and duodenum decreased after various periods of vagal stimulation. After 15 minutes of stimulation, the antrum showed a statistically significant decrease of 22.7% and after 30 minutes of stimulation the duodenum showed a significant decrease of 34.9% (Fig. 5 bottom).

3) Fluorescent histochemical findings

After five minutes of vagal stimulation, yellow 5-HT fluorescence corresponding to the EC cells of the mucosal layer could be seen, but it was much less intense than that in the

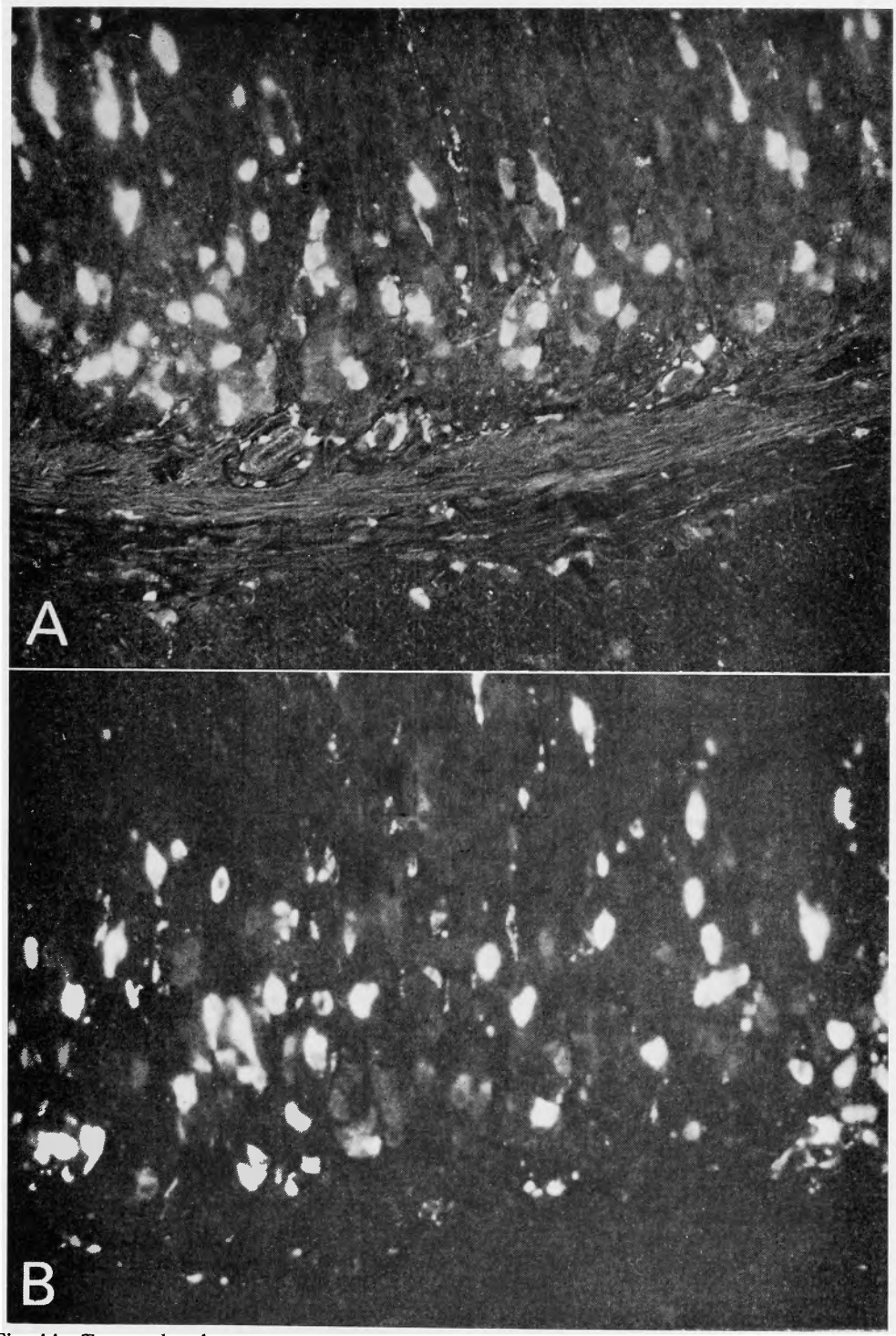

Fig. 4A Two weeks after vagotomy, the yellow 5-HT fluorescence of the mucosal layer was conspicuously increased, and the entire mucosa seemed brighter.

Fig. 4B Three weeks after vagotomy, 5-HT fluorescence corresponding to the EC cells of the mucosal layer was clearly increased.

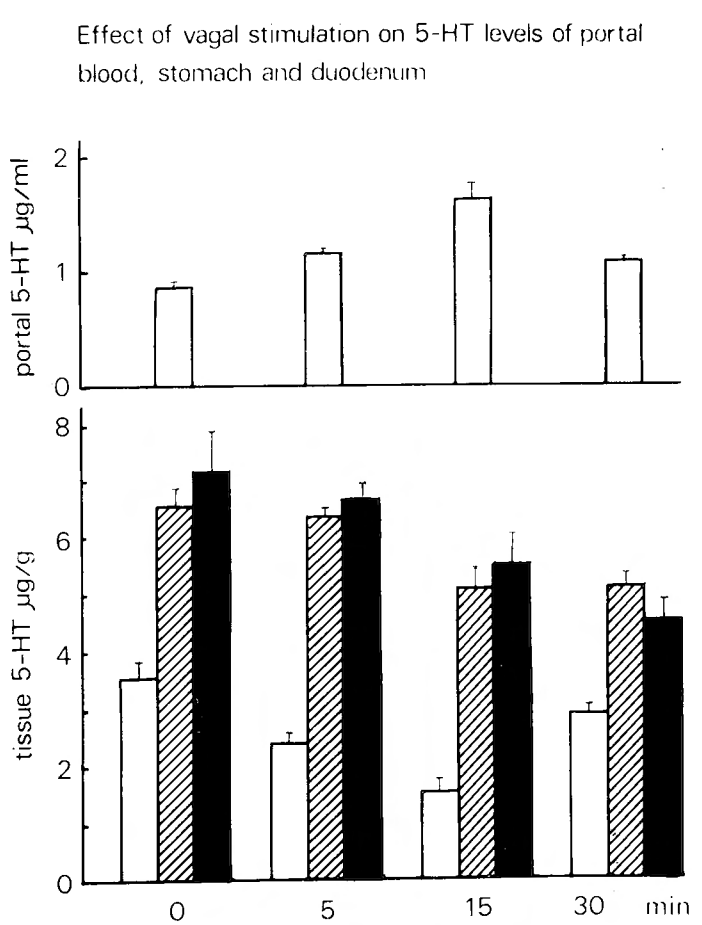

Fig. 5 Top The 5-HT level of portal vein blood increased with electrical vagal stimulation. The 5-HTlevel in portal vein blood was highest after 15 minutes of electric vagal stimulation, or 86.5% above that in the control rats.

Fig. 5 Bottom The 5-HT levels of the autrum (line bars) and duodenum (Black bars) in control rats were, respectively, 6.56 ± 0.32 μg/g and 7.13 ± 0.27 μg/g, approximately twice the level in the corpus (white bars).

The 5-HT levels in the antrum and duodenum decreased after various periods of vagal stimulation. After 15 minutes of stimulation, the antrum showed statistically a significant decrease of 22.7% and after 30 minutes of stimulation the duodenum showed a significant decrease of 34.9%.

control rats. Below the mucosa, dilatation of blood vessels and congestion was seen. The green CA fluorescence surrounding the arteries was not changed (Fig. 6A).

After 15 and 30 minutes of stimulation, the 5-HT fluorescence of the EC cells was markedly less than in the control rats, and even less than after five minutes of stimulation. No noticeable change was seen in the CA fluorescence of the submucosa (Fig. 6B, 6C).

After 15-30 minutes of stimulation, there was a slight change which was thought to be yellow 5-HT fluorescence secreted into the gastrointestinal lumen from the EC cells.

The EC cells are endocrine cells and 5-HT is thought to be internally secreted, but these fluorescent histochemical findings showed that it is also externally secreted (Fig. 7).

The preceding fluorescent histochemical findings correspond well with the results of the chemical assays.

These results suggest that the increase in 5-HT levels in portal vein blood following vagal stimulation is due to the release of 5-HT from the EC cells of the stomach and duodenum.

3. Effect of Various Autonomic Drugs on Changes in Portal Vein Blood and Gastroduodenal 5-HT Levels Caused by Vagal Stimulation

1) Effect of hexamethonium

The 5-HT level of portal vein blood in control rats was 0.86 ± 0.03 μg/ml, but 30 minutes after the intra-peritoneal injection of 5 mg/kg of hexamethonium, which is a ganglion blocker, the 5-HT level was 0.62 ± 0.01 μg/ml, 28% lower than in the controls. After the injection of 5 mg/kg of hexamethonium and 15 minutes of vagal stimulation the 5-HT level of portal vein blood was 0.88 ± 0.21 μg/ml, approximately 29% higher than that of rats non-vagotomized injected with hexamethonium, and nearly the same as that in the control rats. In the control rats, the portal vein blood 5-HT level was raised approximately 87% by vagal stimulation ; thus the release of 5-HT by vagal stimulation was markedly

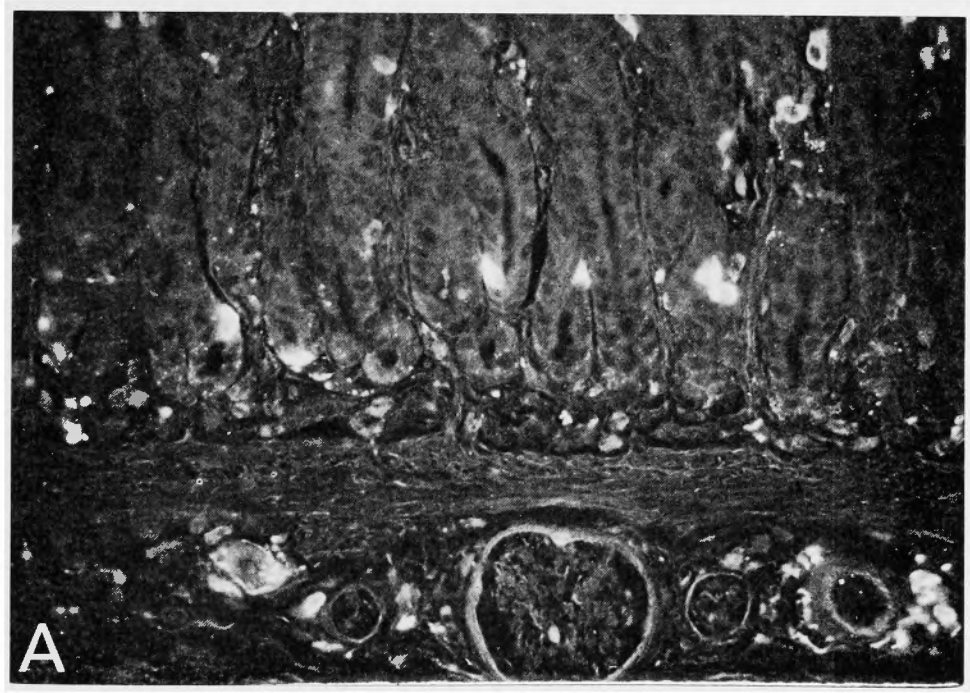

Fig. 6A The 5-HT fluorescence of the mucosal layer decreased conspicuously 5-minutes after electrical vagal stimulation. Dilation and congestion of blood vessels in the submucosal layer can be seen. There were no changes in the CA fluorescence surrounding the artery.

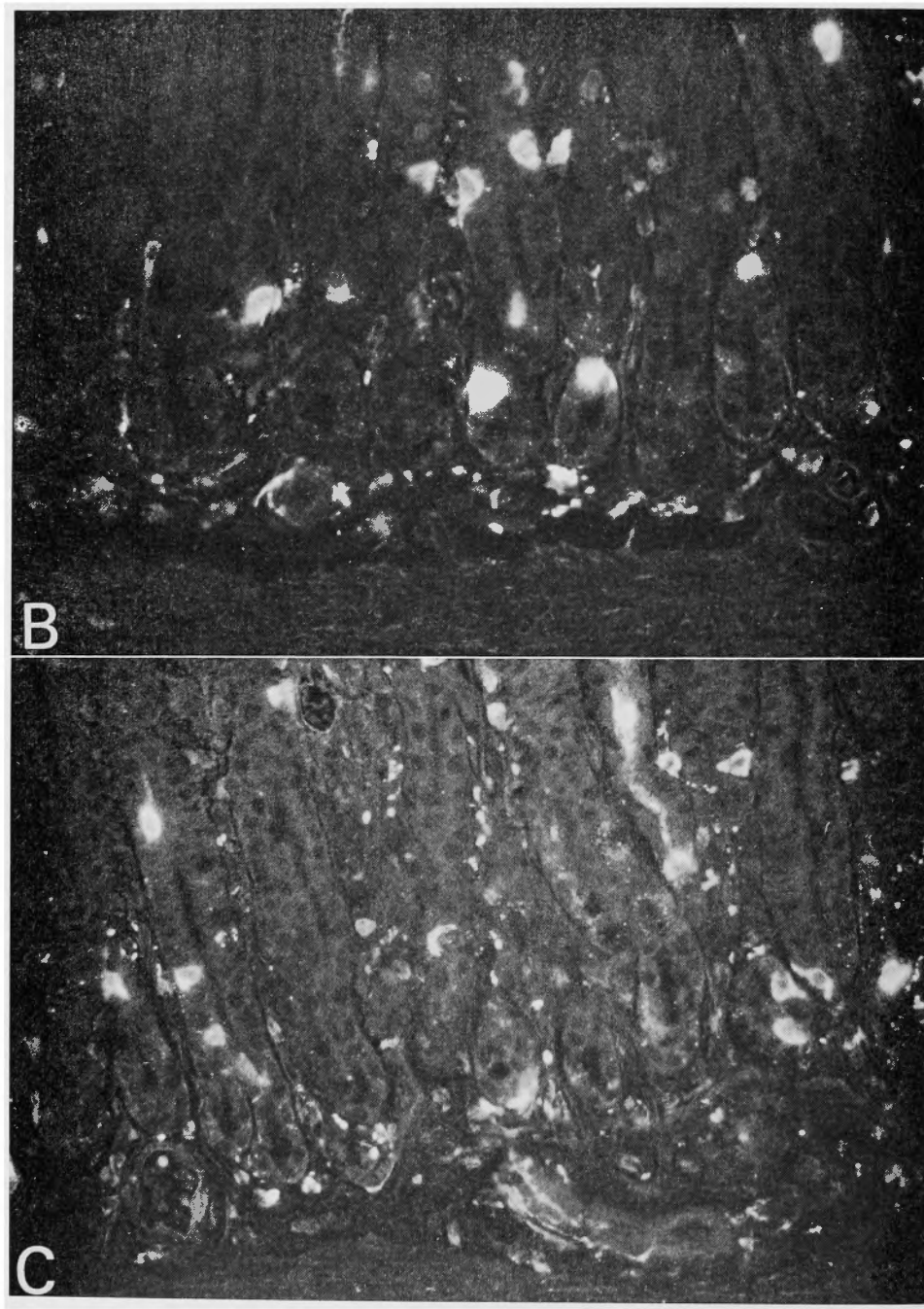

Fig. 6B Fifteen minutes after electrical vagal stimulation, the 5-HT fluorescence of the mucosal layer has decreased conspicuously.

Fig. 6C Thirty minutes after electrical vagal stimulation, there are no changes in the CA fluorescence surrounding the blood vessels, but the 5-HT fluorescence of the mucosal layer has decreased.

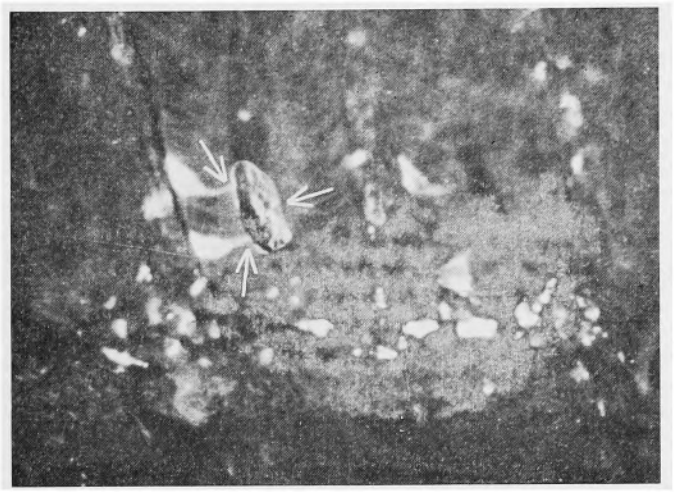

Fig. 7 After 15–30 minutes of stimulation, then was a slight change which was thought to be yellow 5-HT fluorescence secreted into the gastrointestinal lumen from the EC cells. The EC cells are endocrine cells and 5-HT is thought to be internally secreted, but these fluorescent histochemical findings showed that it is also externally secreted (↑).

inhibited by autonomic ganglion blockade.

2) Effect of neostigmine

The 5-HT level in the portal vein blood in control rats was 0.86 ± 0.03 μg/ml, but 30 minutes after the intra-peritoneal injection of 0.1 mg/kg of neostigmine, the level rose to 1.21 ± 0.03 μg/ml, 29% higher than in the control rats. In other words, the inhibition of acetylcholine esterase by neostigmine, strengthened and prolonged the action of acetylcholine which probably raised the 5-HT level in portal vein blood. Thirty minutes after the injection of 0.1 mg/kg of neostigmine, rats were given 15 minutes of vagal stimulation, and the 5-HT level in portal vein blood was 1.27 ± 0.03 μg/ml, almost the same as before stimulation.

3) Effect of atropine

(i) 5-HT in portal vein blood

The fact that 5-HT is released by vagal stimulation and inhibited by autonomic ganglion blockade was clear. The next study was to determine whether 5-HT release is inhibited by a blocker of parasympathetic receptors, atropine. For this purpose, a small enough dose of atropine not to cause ganglion blockade was used. Thirty minutes after the injection of 0.01 mg/kg of atropine, the 5-HT level of portal vein blood was 0.86 ± 0.05 μg/ml, identical with the level in control rats. In rats given 15 minutes of electric vagal stimulation 30 minutes after the injection of 0.01 mg/kg of atropine it was 1.17 ± 0.06 μg/ml. Whereas the 5-HT in portal vein blood in control rats increased 87% after electric vagal stimulation, in those treated with atropine, it increased only 36%. So atropine appears to counteract somewhat the effect of electric vagal stimulation.

Table 3. Effect of Various Autonomous Nervous Drugs on the Changes in 5-HT Levels in Portal Vein Blood by Electrical Vagal Stimulation.

The 5-HT level of portal vein blood after intra-peritoneal injection of 5 mg/kg of hexamethonium has decreased approximately 28% compared to the control group. After administrating hexamethonium, 15 minutes of electrical vagal stimulation was performed and the level (compared with before stimulation) increased approximately 29%, but this level is proportionate to the 5-HT level in portal vein blood of control rats. 5-HT release by vagal stimulation is conspicuously inhibited by hexamethonium.

The 5-HT level of portal vein blood after intra-peritoneal injection of 0.1mg/kg of neostigmine increased approximately 29% compared to the control group. After administrating neostigmine, electrical vagal stimulation was performed, but there was nosignificant difference in the 5-HT level of portal vein blood compared with before stimulation.

There was no change in the 5-HT level of portal vein blood after intraperitoneal injection of 0.01 mg/kg of atropine compared with the control group. After administrating atropine, electrical vagal stimulation was performed and compared with before stimulation, the level increased approximately 36%, but compared with the rate of increase of the control group, this was small.

The 5-HT level of portal vein blood after intra-peritoneal injection of 200 mg/kg of 6-OH-DA increased approximately 64% compared to the control group. After administrating 6-OH-DA, electrical vagal stimulation was performed, but there was no significant increase compared with before stimulation.

Twenty rats were used for the control group and 10 for each of the various other groups.

Portal 5-HT (μg/ml)

	Normal	Vagal S.
None	0.86±0.03	1.61±0.14
Hexamethonium	0.62±0.01	0.88±0.21
Neostigmine	1.21±0.03	1.27±0.03
Atropine	0.86±0.05	1.17±0.06
6-OH-DA	1.42±0.05	1.30±0.13

(ii) Fluorescent histochemical findings

Fluorescent histology of the antrum 30 minutes after the injection of 0.01 mg/kg of atropine showed yellow 5-HT fluorescence corresponding to the EC cells of the mucosal layer, but this was almost the same as in the control rats. Green CA fluorescence was seen surrounding the blood vessels of the submucosal layer and below the muscularis mucosae, but no significant changes were noted. Fluorescent histology of the antrum after 15 minutes of electric vagal stimulation 30 minutes after the injection of 0.01 mg/kg of atropine showed yellow 5-HT fluorescence corresponding to the EC cells of the mucosal layer which was somewhat less intense than that in atropine-treated rats not given electric vagal stimulation, and somewhat more intense than in those given 15 minutes of electric stimulation without atropine. In other words, the inhibitory action of atropine of vagal stimulation was demonstrated and corresponded well with the 5-HT levels in portal vein blood. Surrounding the arteries below the muscularis mucosae, strong green CA fluorescence appeared.

4) Effect of 6-OH-DA

It is known that the vagus nerve contains adrenergic fibers which are distributed not

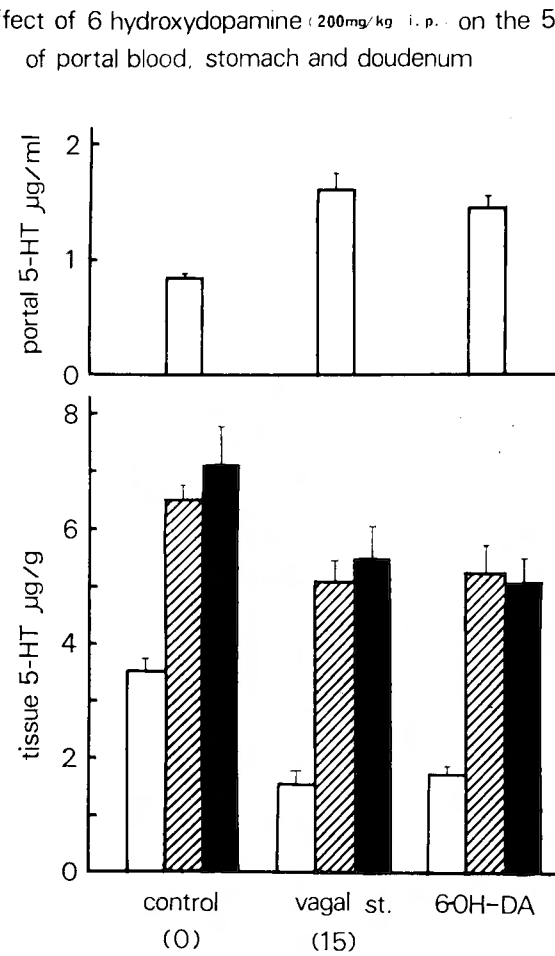

Fig. 8 When 6-OH-DA was administered, the 5-HT level of portal vein blood increased (upper level) and the 5-HT level in the corpus (White bars), the antrum (Lined bars) and the duodenum (Black bars) (in the lower level) all decreased. This is similar to the changes in the 5-HT level of portal vein blood and gastroduodenum after 15 minutes of electrical vagal stimulation.

only to the smooth muscle of the gastrointestinal tract and vascular walls, but also to the parasympathetic ganglia. The 5-HT levels were determined in the gastrointestinal tract and in the portal vein blood of rats after chemical sympathectomy with 6-OH-DA in a study of how adrenergic nerves are involved in 5-HT release during vagal stimulation.

(i) 5-HT in portal vein blood

In control rats, the 5-HT level in portal vein blood was 0.86 ± 0.03 μg/ml, but after 15 minutes of vagal stimulation, it was 1.61 ± 0.14 μg/ml, approximately 87% higher. In rats administered 6-OH-DA, the level was 1.42 ± 0.05 μg/ml, an increase of approximately 64%, this is a significant difference. Vagal stimulation caused no increase of 5-HT in portal vein blood after the injection of 6-OH-DA (Table 3, Fig. 8).

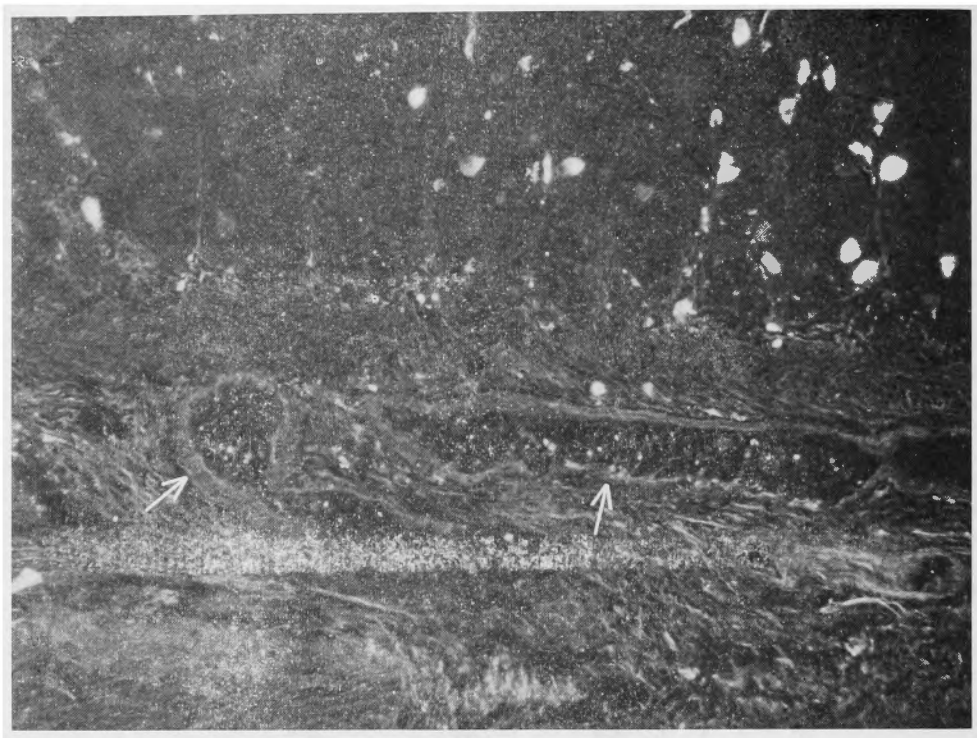

Fig. 9 This is the fluorescence hist ochemical findings of the antrum 1 week after intraperitoneal injection of 200 mg/kg of 6-OH-DA. The blood vessel below the mucosa (↑) has conspicuously dilated and the CA fluorescence in the wall of artery, the muscularis muscosae, within the muscular layer have disappeared or decreased. On the other hand, the 5-HT fluorescence of the mucosal layer has decreased.

(ii) 5-HT in gastroduodenum

Corpus : 5-HT in the corpus of control rats was 3.58 ± 0.34 $\mu g/g$, but after 15 minutes of vagal stimulation it was 1.61 ± 0.21 $\mu g/g$, a decrease of approximately 55%. In rats injected with 6-OH-DA it was 1.78 ± 0.10 $\mu g/g$, approximately 50.3% of that in control rats (Fig. 8).

Antrum : The 5-HT level of the antrum in control rats was 6.56 ± 0.32 $\mu g/g$, but after 15 minutes of vagal stimulation, it was 5.07 ± 0.36 $\mu g/g$, a decrease of approximately 22.0%.

In rats administered 6-OH-DA, the level was 5.11 ± 0.57 $\mu g/g$, decrease of approximately 28% (Fig. 8). Chemical sympathectomy with 6-OH-DA caused changes in the 5-HT levels in portal vein blood and in the duodenum similar to the changes caused by vagal stimulation.

(iii) Fluorescent histochemical findings

Yellow 5-HT fluorescence corresponding to the EC cells of the mucosal layer was seen, but much less than in the control rats. All the blood vessels in the submucosal layer were dilated and the green CA fluorescence usually seen surrounding the arteries and within the muscle layer had entirely disappeared (Fig. 9).

Discussion

Among the chemical stimulants which release 5-HT from EC cells, hypertonic glucose, 0.1 N HCl, and $MgSO_4$ are well known.

The release of 5-HT by local intraluminal stimulation has been proved by many investigators (TOBE, KIMURA and FUJIWARA, 1967).

O'HARA, FOX and COLE (1959) reported that the oral (intraluminal) administration of 50% glucose or sucrose raised 5-HT levels in the portal blood.

DRAPANAS, MCDONALD and STEWART (1962), PESKIN and MILLER (1962) and JOHNSON et al. (1962) reported that the intraluminal administration of hypertonic glucose solution (50% glucose, 150-200ml) into dogs' proximal intestine caused increased 5-HT levels in the portal blood and simultaneously induced different signs comparable with those in human dumping syndrome.

Using fluorescence histochemistry, TOBE et al. (1967) demonstrated a decrease or even disappearance of 5-HT fluorescence in the human jejunum exposed to 50% glucose solution During laparotomy 20ml of 50% glucose solution was administrered directly by a syringe. Thirty min. later a biopsy of the jejunum was taken from the same portion and examined.

We administrated various liquids directly into the duodenum of rats during laparatomy under nembutal anesthesia. Two ml of hypertonic glucose, sucrose and fructose, 0.1 N HCl or 30% $MgSO_4$ solution caused 5-HT release, but physiologic saline solution did not (TOBE, 1973).

Many gastrectomy patients experience the Dumping Syndrome caused by hypertonic glucose. The mechanism of 5-HT release in these cases is that the EC cells protrude microvilli on the gastrointestinal cavity side which contain 5-HT granules in the basement and receive stimulation from the cavity causing release of 5-HT (by emiocytosis) into the basement membran. That this occurs has been confirmed by electron microscope research.

In anesthetized dogs KOBAYASHI and FUJITA (1973) demonstrated by electron microscopy emiocytic release of the 5-HT containing granules of the EC cells in the duodenum after 50% glucose solution was administred intraluminally.

All these findings indicate that 5-HT release can be induced by local intraluminal stimulation of the mucosa. In addition to this mechanism, 5-HT may be released by nervous machanism, which is the main subject of this communication.

The relationship between the vagus nerve and 5-HT has been studied by the research groups of DRAPANAS (1962, 1971) or of STRAUSS (1972). DRAPANAS et al. (1971) reported that 5-HT fluorescence decreased in the rat stomach after vagotomy.

STRAUSS et al. have reported that 5-HT levels in portal vein blood increase conspicuously following electric vagal stimulation in dogs and that when ischemia of the intestinal tract is caused by ligation of the superior mesenteric artery, the 5-HT level in portal vein blood increases conspicuously ; however, in vagotomized dogs the 5-HT level in portal vein blood does not vary much after electric stimulation or mesenteric artery ligation.

The present study shows the involvement of autonomic nerves in the mechanism of 5-HT release by EC cells in rats. In this study, truncal vagotomy performed on rats had almost no effect on 5-HT levels in portal vein blood, but chemical assay and fluorescent histochemical studies showed that gastroduodenal 5-HT levels do increase. LEVINE et al. (1969), however, have reported that in 5-HT assays of the rat alimentary tract from the esophagus to the anus two weeks after vagotomy, the 5-HT level in the stomach and cecum decreased to 1/3 the level in control rats. WEICHERT et al. (1970) also noted that 5-HT fluorescence in the stomach of rats decreased 3-5 days after vagotomy.

In our experiments also, when fasting was incompelete and there was even a small amount of food in the stomach, the gastroduodenal 5-HT levels was often decreased. The present writer is the only one to report that gastrointestinal 5-HT levels increase conspicuously after vagotomy. After vagotomy, if pyloroplasty has not been performed, food tends to accmulate in the stomach, and it is presumed that pressure within the stomach increases and that physical and chemical stimulation in the gastrointestinal cacity causes 5-HT to be released from the EC cells.

The preceding findings suggest that 5-HT release from the EC cells is inhibited by vagotomy. To confirm the effect of vagus nerve innervation on 5-HT release from the EC cells, electric vagal stimulation was performed.

Chemical assay and fluorescent histochemical experiments confirmed that electric vagal stimulation conspicuously increased 5-HT levels in portal vein blood and decreased gastroduodenal 5-HT levels. Other experimental results also suggest that the vagus is involved in 5-HT release. For example, after truncal vagotomy, the dumping syndrome, which is said to be caused by 5-HT release, occurs rarely. Tobe et al. noted that after anesthesia of the intestinal tract mucosa, when hypertonic glucose is administred, 5-HT levels in portal vein blood show almost no change and that the mechanism of 5-HT release is blocked and does not function. In previous experiments by the present writer, when hypertonic glucose was injected into the stomachs of vagotomized rats, a decrease in the 5-HT level in the antrum and a conspicuous increase in 5-HT in portal vein blood were seen. It is thought that this is because 5-HT release from the EC cells is inhibited by vagotomy, but because synthesis continues, 5-HT in the gastrointestinal tract increases. However, chemical stimulation, such as by hypertonic glucose in the gastrointestinal cavity, causes accumulated 5-HT to be released and the 5-HT level in portal vein blood to rise conspicuously.

Our results have made it clear that 5-HT release is inhibited by section of the vagal nerves and is promoted by vagal stimulation. Fluorescent histochemical procedures have shown that the vagal nerves contain both parasympathetic and sympathetic fibers. Furthermore, it is certain that parasympathetic preganglionic fibers are cholinergic. There is some evidence that there are non-cholinergic postganglionic fibers as well as cholinergic fibers in the smooth muscle of the alimentary tract. BURNSTOCK (1972) proposes a purinergic nerve and BÜLBRING and GERSHON (1966, 1967) advocate 5-HT involvement.

In this experiment also, the effect of various autonomic drugs on 5-HT release by vagal

stimulation was examined. 5-HT release by vagal stimulation was conspicuously inhibited by the administration of 5 mg/kg of hexamethonium, a nerve ganglion blocker. The 5-HT level in portal vein blood was increased approximately 29% by the administration of 0.1 mg/kg of neostigmine, but no further increase was caused by vagal stimulation. When 10 mg/kg of atropine was administred to block the cholinergic muscarinic receptor, 5-HT release by vagal stimulation was partially inhibited. These findings make it clear that there is a 5-HT release which involves cholinergic fibers, but there may also be a non-cholinergic component. To clarify this point further studies are necessary.

BÜLBRING et al. also report that 5-HT release is inhibited by the administration of atropine, hexamethonium and procaine. On the assumption that there is a 5-HT release which involves parasympathetic nerves, the following experiment was designed to determine whether parasympathetic ganglion-innervated noradrenergic fibers in the alimentary tract are involved in 5-HT release. Since 6-OH-DA is taken up selectively by noradrenergic nerves and causes changes in the nerve terminals, it is used for the purpose of chemical sympathectomy (THOENEN et al., 1967 ; TRANZER and THOENEN, 1968 ; THOENEN and TRANZER, 1968).

One week after the administration of 6-OH-DA, complete noradrenergic denervation can be seen by fluorescent histochemical procedures. By this time, the 5-HT level in portal vein blood has increased conspicuously and gastrointestinal 5-HT has decreased. This condition is similar to that in rats administred neostigmine or given vagal stimulation, and it is thought that the noradrenergic fibers function to inhibit 5-HT release by vagal stimulation, probably because of the similarity of cholinergic fibers in the smooth muscle of the alimentary tract to noradrenergic fibers.

Gastrin is the gut hormone which has been investigated most thoroughly in respect to its relationship to the vagus nerve, and the concept of the vagal release of gastrin was established by UVNÄS (1942) and OLBE et al. (1966).

It can be conclusively stated that 5-HT, as gastrin, may be released by two mechanism One is a response to intraluminal stimulation of the mucosa. The other is mediated by vagal stimulation, i. e., the vagal release of 5-HT. The latter is mediated by cholinergic fibers, while adrenergic fibers inhibit 5-HT release.

The main purpose of treating peptic ulcers, surgically is to decrease the secretion of gastric juice. There are two major types of surgery : vagotomy, which severs the vagal pahse, and gastrectomy, which severs the gastric phase of gastric jujice secretion. In Europe and America, vagal nerve section is often done for duodenal ulcers and in Japan the operative methods for treating peptic ulcers are now being re-examined. There are still many unknown points in the physiological effect of vagotomy. The present study provides important background information which should help to crlarify the uses of vagotomy.

Conclusions

1) When the vagal nerves of rats were severed, there was almost no change in the

5-HT level of portal vein blood ; gastrointestinal 5-HT levels did not decrease, but, rather, showed a tendency to increase and starting two weeks after vagotomy, the gastroduodenal 5-HT level increased significantly. Fluorescent histochemical studies also showed that the 5-HT level of the antrum increased conspicuously.

2) When electric vagal stimulation was performed on rats, the 5-HT level in portal vein blood increased conspicuously (approximately 86.5%) and the gastroduodenal 5-HT level clearly decreased. Fluorescent histochemical studies also showed that the 5-HT level in the antrum decreased conspicuously.

3) After the intramural ganglia had been blocked by the administration of hexamethonium, electric vagal stimulation caused almost no changes in the 5-HT level of portal vein blood.

4) When neostigmine was administered, the 5-HT level of portal vein blood clearly increased (28.6%). After the administration of neostigmine, further electric vagal stimulation caused no further increase in the 5-HT level of portal vein blood.

5) After chemical parasympathectomy by atropine, electric vagal stimulation caused only a small amount of increase in portal vein blood. Fluorescent histochemical studies showed that the 5-HT level in the antrum did not decrease much and the cholinergic nerve fibers were inhibited by atropine.

6) When chemical sympathectomy was performed by 6-OH-DA, the 5-HT level in portal vein blood increased conspicuously (64.4%) and the gastroduodenal 5-HT level decreased conspicuously. A decrease of 5-HT in the antrum was also shown by fluorescent histochemical procedures, and catecholamine fluorescence disappeared completely. In other words, it is thought that blockade of adrenergic fibers caused cholinergic fibers to function predominantly and release 5-HT.

7) These findings suggest that 5-HT in the gastrointestinal tract can be released apart from adequate stimulation from within the gastrointestinal cavity, by the action of the vagal nerves to stimulate the release of 5-HT. This release seems to involve cholinergic fibers, and adrenergic fibers inhibit the process.

The gist of the present study was reported at the 59th, 60th, and 61st General Meeting of Japanese Gastroenterologic Society and Vth Wordl Congress of Gastroenterology, Mexico, 1974.

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和文抄録

ラットにおける胃腸セロトニンの放出機序に関する研究

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ラットの迷走神経を切断すると，門脈血中の 5-HT はほとんど変化がないが，胃十二指腸の 5-HT は減少することなく，むしろ増加傾向を示し，vagotomy 2週間後より有意差をもって胃十二指腸の 5-HTは増量した．螢光組織化学的検索でも胃幽門洞部の 5-HT は著明に増量した．

ラットの迷走神経を電気刺激すると，門脈血 5-HT は著明に増量（約86.5%）し胃十二指腸の 5-HTは明らかに減量した．螢光組織化学的検索でも胃幽門洞部の 5-HT は著明に減少した．

Hexamethonium を投与して壁内神経節を遮断してから，迷走神経を電気刺激しても門脈血中の 5-HT はほとんど増量しなかった．

Neostigmine を投与すると門脈血中の 5-HT は明らかに増量（28.6%）した．Neostigmine 投与後，さらに迷走神経を電気刺激しても門脈血中の 5-HT は Neostigmine 投与により増量した以上に増量しなかった．

Atropine を投与して chemical parasympathectomy を行なってから，迷走神経を電気刺激しても門脈血 5-HT は，わずかしか増量せず，螢光組織化学的にも胃幽門洞部の 5-HT はあまり減少せず，Atropine により cholinergic nerve fibers が抑制された．

6-Hydroxydopamine を投与して chemical sympathectomy を行なうと，門脈血 5-HT は著明に増量（64.4%）し，胃十二指腸の 5-HT は著しく減量した．螢光組織化学的にも胃幽門洞部 5-HT の減少がみられたが，catecholamine 螢光は，まったく消失した．すなわち adrenergic nerve fibers の遮断により cholinergic nerve fibers が優位に作動し，5-HT の release をきたしたものと考えられる。

以上の所見は，胃腸管の 5-HT が管腔からの適正刺激による放出以外に迷走神経性放出の関与，すなわち vagal release of 5-HT の存在を示唆するものであり，この放出は，cholinergic nerve fibers を介すると考えられ，adrenergic nerve fibers は抑制的に働くものと考えられる．