charged salicylate ions will be attracted and adsorbed onto the positively charged micelles in such a manner of arrangement that gives rise to an increase in size and thereby to an increase in viscosity. As more acid is added more salicylate ions will be adsorbed on the micellar surface and this continues till a maximum viscosity is reached. At this stage the micellar surface may have adsorbed sufficient salicylate ions to attain an equilibrium state so that further additions of the acid will tend to upset the equilibrium resulting in a decrease in viscosity. When the amount of acid present exceeds that which can be solubilized by the micelles then the solution or gel becomes a suspension of salicylic acid in the surfactant solution. This change of nature of the system makes it no longer a simple liquid system and this gives rise to non-Newtonian flow which is not unusual with the flow properties of most suspensions. In the case of the alkyl pyridinium compounds the flow properties remain non-Newtonian in the presence of an excess amount of salicylic acid. This is also true for the alkyl trimethyl ammonium compounds studied previously (7).

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Keyphrases

Salicylic acid–quaternary ammonium compounds—interaction
Viscosity—salicylic acid–quaternary ammonium compounds
Rheology—salicylic acid–quaternary ammonium compounds

Effect of Vagal Stimulation on Enterochromaffin Cell Granulation in the Guinea Pig Small Intestine

By MARTIN F. TANSY, ARTHUR S. MILLER, and ARTHUR STEIN

Experiments are presented which study the effect of vagal stimulation on the enterochromaffin cell granulation of the guinea pig duodenum. Concentration of granulated enterochromaffin cells in specially stained intestinal sections is used to determine the response. The results indicate the vagus nerves *per se* and not hydrochloric acid secretion, increased intraluminal pressure, or hyperperistalsis significantly alter the granulation of these cells. The data also suggest that vagal degranulation may occur *via* a noncholinergic mechanism.

THE IMPORTANCE of serotonin in the gastrointestinal tract has received considerable investigative attention. Most of the studies, however, have been concerned with its content, fate, and action; whereas, its release has received much less attention. Serotonin release has primarily been concerned with the observations that elevation of intraluminal pressure and/or increased intestinal motility augments the amount of 5-HT released into the intestinal lumen and venous blood (1). It has not, however, been shown that distension or hyperperistalsis of the intestine, kept within physiologic limits, produces in the intact animal a local or systemic discharge of serotonin from the enterochromaffin cells (2).

Argentaffin enterochromaffin cells are distributed throughout the mucosa of the gastrointestinal tract. They contain specific granules which are precipitated by formalin and will stain with silver salts (3). The complete chemistry of the granules is not known, but Barter and Pearse (4) suggest a fully conjugated β-carboline derivative of serotonin may precipitate the silver to give the argentaffin staining reaction. In any event, considerable experimental evidence (5–7) has shown that a decrease in the granularity of these cells parallels the release of serotonin from the gut. This phenomenon provides a convenient

Received May 22, 1968, from Temple University Schools of Dentistry and Pharmacy, Philadelphia, PA 19140
Accepted for publication July 22, 1968.
This investigation was supported by grant AM-11372-01 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
The authors wish to express their appreciation to Mrs. N. C. Gulezian for her assistance in preparation of the histologic material, and to Mr. Alex Mucha for his help in preparing the photographs.

histologic method for studying gastrointestinal serotonin release.

The present study was designed to provide information on the effects of vagal stimulation on the granulation of the enterochromaffin cell system as a link to serotonin release in the bowel.

EXPERIMENTAL

Animals—Acute experiments were performed on 76 healthy male guinea pigs, weighing 350–450 g., aged about 6 months. The strain was random bred from closed colonies obtained from the Marland Farms, Wayne, N. J. The guinea pigs were kept in the laboratory for an acclimatization period of 3 days. Housing was in cages accomodating six animals. In addition to standard stock commercial pellet food, they were given "greens" and fresh tap water *ad libitum*. All animals were fasted but allowed water 24 hr. before operation.

Selection of the male guinea pig was for several reasons: (*a*) There is only one principal type of enterochromaffin cell in the alimentary tract of the guinea pig, the argentaffin cell (8). (*b*) The guinea pig has five to eight times as many argentaffin enterochromaffin cells in the proximal part of the duodenum as the rat (9, 10). (*c*) There are fewer mast cells in guinea pig intestine compared to the greater number found in rat and mouse bowel. (*d*) It is generally agreed the argentaffin reaction is the most convenient and suitable means for demonstration of guinea pig enterochromaffin cells (11–13). In this animal, these cells exhibit an intense argentaffin reaction compared to the weak reaction intensity of similar cells in other rodents. (*e*) Males were used because even in the same species, individual enterochromaffin cell counts vary depending on the animals' sex. A greater number of enterochromaffin cells has been found in the adult female guinea pig than in the male (14–17). It was pointed out, however, that the enterochromaffin cell count varies with the stage of the estral cycle (17).

Surgical and Recording Procedures—Under intraperitoneal urethan anesthesia (1.5 g./kg.), a midline abdominal incision was made and both branches of the subdiaphragmatic vagus isolated and secured to stimulating electrodes. In these laparotomized animals an opening was made in the stomach and jejunum, and in some animals the common bile duct was also ligated. These procedures were effected to minimize the effects of gastric juice acid, intraluminal pressure changes, and bile on the duodenal mucosa. In other animals the vagi were exposed, divided, and stimulated high in the neck. Peripheral vagal stimulation at the cervical level was carried out with the nerves either intact or cut. The external jugular vein was cannulated for administration of drugs. A tracheotomy was established in all animals for either recording intratracheal pressure with a volumetric transducer (Statham PT 5A) or maintaining artificial respiration. Right carotid arterial pressure was recorded continuously using a pressure transducer (Statham P23 AC). Scalar lead II of the electrocardiogram and tachograph was also recorded. All variables were charted on a polygraph (Grass model 7).

Electrical Stimulation—Bipolar electrodes were attached to the caudad portion of the vagus nerves and surrounded with liquid petrolatum to isolate the stimulating current. The vagi were stimulated with a stimulator (Grass model S8) *via* an isolation transformer. The stimulus was applied for a total of 15 min., 5 min. on, 5 min. off. This stimulus pattern delivered at the nerve site was monitored with an oscilloscope (Tektronix 564). The voltage was varied between 1 and 10 v. depending on response of the organ to nerve stimulation. Stimuli were applied at frequencies varying from 1–250 c.p.s./sec. Unless otherwise indicated, duration of the pulse was kept constant at 1 msec. throughout all experiments. In some cases, the duodenal musculature was stimulated directly using the same stimulus pattern. Control animals were similarly anesthetized, laparotomized, and remained on the operating table for the same period of time.

Histology—The guinea pigs were sacrificed by a Harvard small animal decapitator. The pyloric antrum, gall bladder with biliary tree, and duodenum were immediately excised, rinsed in Tyrodes, and fixed in 10% formalin. After 24 hr. fixation, sections of proximal, intermediate, and distal duodenum were selected and trimmed to about 4 mm. in length. Every attempt was made to consistently define from animal to animal, the precise anatomic tissue selection site for histologic evaluation. The proximal portion of duodenum was defined as that segment *juxta* the pylorus situated between the pyloric ring and entrance of the common bile duct. Selection of middle and distal portions of duodenum, however, tended to be more subjective due to absence of gross anatomical landmarks. From each specimen 2 to 3 transverse paraffin sections were cut at 6 μ. Sections were stained by the Fontana-Masson technique, counterstained with eosin, and mounted in a fixative (Permount).

Cell-Counting Technique—To give numerical results, argentaffin cells in each specimen were counted in adjacent fields at 430X using a light microscope. This method of determining the number of argentaffin cells in terms per visual field of stated size has been successfully employed in a number of studies (5, 18, 19). As a point of reference each section was adjusted so that musculature was always present in the microscopic field. The distribution of argentaffin cells is not uniform over the mucosa. The heaviest concentrations are found in the basal area, therefore, tangential sections were rejected as such fields contained a disproportionate amount of basal area. The number of cells in each tissue was expressed as cells per high-power field (H.P.F.). Up to 40 fields in each section were counted depending on circumference of tissue available. The mean number of cells/H.P.F. was calculated for the three specimens of duodenum in each animal. All sections were judged under blind conditions. The t test was used to determine the significance of differences between mean values of all reported data. P values are indicated where applicable.

Drugs—Each of 12 guinea pigs received an intravenous injection of atropine sulfate, six at a dose of 5 mg./kg., the remainder at 10 mg./kg. *via* the external jugular vein immediately before surgical exposure and mechanical stimulation of the cervical vagi. Four atropine controls were also employed. To ascertain if this method was sensitive enough to

detect changes in enterochromaffin cell granulation, reserpine[1] was administered to 6 animals. A single injection of reserpine (30 mg./kg.) was given intraperitoneally after the volume of the solution was adjusted to 4.0 ml. with warm saline. A comparable number of control animals received a similar volume of physiologic sodium chloride at 37°. Twenty-four hours postinjection, the reserpinized animals exhibited a 90% decrease in their mucosal granulated argentaffin cell count. This acknowledged chemical degranulator served as an effective technique control.

RESULTS

The results of these experiments indicate that the distribution of argentaffin enterochromaffin cells within the guinea pig duodenum varies considerably. The most consistent distribution as well as the greatest number of cells was found in the section of duodenum between pyloric ring and opening of the common bile duct. In some animals, however, peak levels of density occurred in the middle third of the duodenum. The lowest number of cells was always found in the distal segment.

Regardless of sectional origin, enterochromaffin cells were most numerous in the basal mucosa, especially in the crypts of Lieberkühn. Occasionally individual enterochromaffin cells were found in the villi and/or submucosa. No stained enterochromaffin cells were detected in musculature. The argentaffinity also varied considerably from animal to animal but no general difference was noticed between the villous and basal enterochromaffin cells in staining intensity. For the most part, enterochromaffin cells were easily distinguishable from other intestinal cells and hemosiderin-laden macrophages. The surgical procedure had no discernible effect on the enterochromaffin cell population.

Under the conditions of this experiment, peripheral vagal stimulation at either 10 v., 25 c.p.s., or 1 v., 250 c.p.s. for 1 msec. produced a significant reduction in argentaffin-positive enterochromaffin cell granularity in guinea pig duodena (Fig. 1). The percentage reduction of granulated argentaffin-positive cells with current of low intensity (1 v., 1 msec., pulse at 250/sec.) ranged from 60 to 91% with a mean reduction of 73% at the $p < 0.005$ level, compared with control animals. At increased current strength (10 v., 1 msec., pulse at 25/sec.), the percentage degranulation ranged from 22 to 82% with a mean reduction of 60% which proved highly significant. A 50% reduction of cell granularity was noted in those animals which were subjected to direct electrical stimulation at low voltage, high frequency stimulus parameters, to their duodenal musculature.

As previously reported (20), the administration of atropine sulfate (5–10 mg./kg.) by itself had no effect on the enterochromaffin cell granulation. The argentaffin degranulation which had been obtained in control animals upon peripheral vagal stimulation was not abolished by atropine at any dose, even though the cholinergic cardiovascular responses to cervical vagal stimulation were completely blocked. The results of vagal stimulation under these various conditions are summarized in Fig. 2.

[1] Serpasil, Ciba Pharmaceutical Co., Summit, N. J.

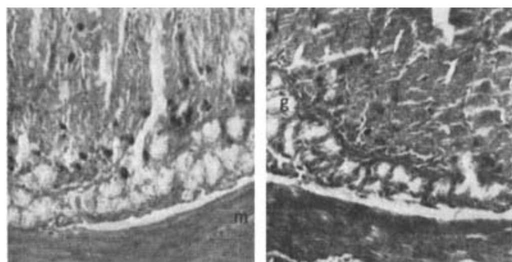

Fig. 1—Photomicrographs of a representative control section (left) and a section from a vagally-stimulated animal (right). Greater numbers of argentaffin cells are apparent in the control section adjacent to Brunner's glands (g). (m) indicates the tunica muscularis (Fontana-Masson stain, ×100).

DISCUSSION

Many of the observations on the distribution of enterochromaffin cells in this study are in agreement with earlier findings (9, 21–23). Hoeschen (9) also found guinea pig enterochromaffin cells were most numerous in that portion of duodenum adjacent to the pylorus and that the count markedly decreased in the distal part of this segment. This segmental gradient has too often been overlooked in interpreting changes in enterochromaffin cell granulation evoked by electric and pharmacologic stimuli.

Routine histologic examination of guinea pig duodena showed enterochromaffin cell granularity to be decreased following peripheral vagal stimulation. The effects of vagal stimulation on enterochromaffin cell density appear to depend on strength, duration, frequency, and mode of application of the stimulus. The degree of degranularity may be frequency-dependent. In any event, vagal degranulation was not attributable to changes in intraluminal pressure because the stomach and

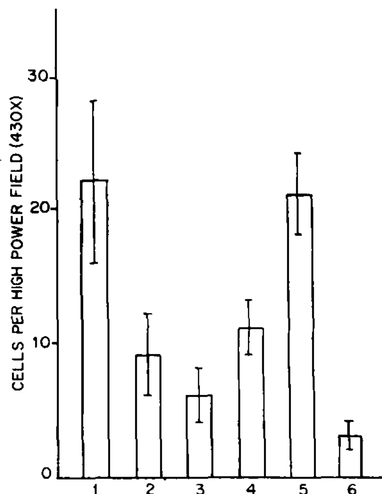

Fig. 2—Bar 1: controls; Bar 2: abdominal vagal stimulation with relatively high voltage, low frequency; Bar 3: abdominal vagal stimulation with low voltage, high frequency; Bar 4: direct electric stimulation to duodenal musculature; Bar 5: atropinized controls (5–10 mg./kg.); Bar 6: cervical vagal stimulation in the atropinized animal. Each bar represents the mean value ± standard deviation.

jejunum were opened at the time of stimulation, thereby negating any rise in pressure. The influence of any major endocrine system on enterochromaffin degranulation seemed unlikely because of the relatively short period and pattern of stimulation used.

Visual inspection of the atropinized animals' small intestine revealed an absence of spontaneous motor activities. In fact, during the stimulus interval, the small intestine remained atonic, flaccid, and completely quiescent. However, animals who had direct electric current applied to their duodenal musculature, exhibited increased intestinal motor activities, but the augmented motility was not as effective in degranulating enterochromaffin cells as was nerve stimulation. These observations led to the conclusion the vagus nerves *per se* and not hydrochloric acid secretion, increased intraluminal pressure, hyperperistalsis, or bile release altered the granularity of the enterochromaffin cells of the guinea pig small intestine.

Even more surprising was the fact that although atropine blocked the cardiovascular parameters to cholinergic stimulation, it failed to block the enterochromaffin cells from discharging their granules on cervical vagal stimulation. Straub and Stefansson (26) have shown convincingly in the guinea pig that stimulation of the vagus *in vivo* evokes small intestinal peristalsis which is easily blocked by atropine, even in doses as small as 10 mcg./kg. Assuming an even distribution of the drug, this would represent a concentration of 10^{-8}, which is in reasonable agreement of atropine required to block acetylcholine contractions on guinea pig ilea *in vitro* (27). In view of this, plus the high dosage level (10 mg./kg.), it seemed unlikely that atropine failed to block enterochromaffin degranulation to vagal stimulation due to acetylcholine release in an area where atropine was unable to penetrate. Furthermore, difficulty of access was not likely to be the answer here because the motor response of the bowel to peripheral vagal stimulation appeared to have been successfully blocked, suggesting that atropine had penetrated at least to the site of nervous action in the muscle. If, on the other hand, the neural pathway controlling enterochromaffin cell degranulation is acetylcholine mediated, it is certainly resistant to antagonism with atropine. Perhaps a more likely explanation is that vagal stimulation affects the enterochromaffin cell granulation in the guinea pig small intenstine *via* a noncholinergic mechanism.

In summary, even though it was found adequate electrical stimulation of the vagus nerve does alter granulation of guinea pig enterochromaffin cells, more work is needed to define the nature of the nerve fibers coursing in the vagus responsible for degranulation. Furthermore, even though the present experiments support this concept of serotonin release by vagal stimulation, at least as measured by enterochromaffin granulation, it remains to be seen if degranulation of enterochromaffin cells can be directly correlated with decreased tissue levels of serotonin under the conditions set forth in these experiments.

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Keyphrases

Enterochromaffin cell granulation—guinea pig duodenum
Vagal stimulation—enterochromaffin cell granulation
Atropine—enterochromaffin cell granulation
Reserpine—enterochromaffin cell granulation