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ACKNOWLEDGMENTS AND ADDRESSES

Received July 20, 1970, from the *Department of Pharmacology' College of Medicine, University of Iowa, Iowa City, IA 52240*

Accepted for publication September 30, 1970.

This study was supported by U. S. Public Health Service Grant NB-02928.

A preliminary report of a part of these experiments appeared in: *Pharmacologist*, **8**, 206(1966); and *Fed. Proc.*, **27**, 754(1968).

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# Effect of Vagal Stimulation on Duodenal Serotonin in the Guinea Pig

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**Abstract** □ The serotonin concentration of duodenal tissue was examined immediately after peripheral or central vagal stimulation, and an 18 or 36% reduction of serotonin, respectively, was found. This is the first demonstration of a decrease in gastrointestinal serotonin in response to autonomic nerve stimulation.

**Keyphrases** □ Serotonin, duodenal—effect of vagal stimulation, guinea pig □ Gastrointestinal serotonin, reduction—response to autonomic nerve stimulation □ Spectrophotofluorometry—analysis

Enterochromaffin cells (EC) are found throughout the gastrointestinal tract, but in the guinea pig their greatest concentration is in the duodenum, *juxta* the pyloric sphincter (1, 2). They are most frequently found deep in the crypts of Lieberkuhn and can be identified by staining the cell granules with silver salts after precipitation by formalin (3). These granules are believed to contain 5-hydroxytryptamine (5-HT, or serotonin), and a high degree of correlation has been reported between EC number and mucosal serotonin concentration in laboratory and domestic animals.

Stimulation of the vagi was shown to result in approximately a 75% decrease in EC counted in the proximal duodenum of the guinea pig (4). Reduction of cells counted merely reflected cellular degranulation, or at least a decrease in stainable substance within the cells. Since serotonin is most frequently identified with these cells, the purpose of this study was to determine if stimulation of the vagi is also associated with a change in tissue serotonin concentration.

## EXPERIMENTAL

**Animals and General Procedure**—Seventy guinea pigs were secured and maintained as previously described (4). Anesthetized animals were divided into three groups. Group I animals (controls) were sacrificed in three subgroups: (*a*) immediately after achieving surgical anesthesia, (*b*) 30 min. later, or (*c*) 60 min. later. Group II animals had the vagi stimulated peripherally and were sacrificed 30 or 60 min. later. Group III animals were sacrificed at 30 or 60 min. after the initiation of central vagal stimulation. In all animals the duodenal tissue *juxta* the pyloric sphincter was removed for spectrophotofluorometric analysis of serotonin according to the method of Wise (5). One group of unanesthetized animals was sacrificed by decapitation. Of these, four received 10 mg./kg. reserpine intraperitoneally 24 hr. prior to decapitation. Results were analyzed for significance at the 0.05 level using a two-tailed Student *t* test.

**Surgical and Recording Procedure**—Under intraperitoneal urethan anesthesia (1.5 g./kg.), the vagi were exposed, divided, and stimulated high in the neck. At the cervical level the vagi were stimulated peripherally or centrally. Carotid arterial pressure was recorded continuously using a Statham pressure transducer. 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 or central portion of the vagi and surrounded with liquid petrolatum to isolate the stimulating current. The vagi were electrically stimulated with a Grass model S8 stimulator *via* an isolation transformer. The stimulator delivered a rectangular pulse (5 v., 30 Hz., 1 msec.), which was applied for a 5-min. duration followed by a rest interval of equal duration for a total of three times.

## RESULTS

The results of this study are found in Fig. 1. The concentration of serotonin in unanesthetized animals was about 4.42 mcg./g. duodenal tissue, whereas after reserpine administration the concentration decreased to 0.43 mcg./g. or by approximately 90%.

Immediately after the guinea pigs were anesthetized, the duodenal tissue had a serotonin concentration of 3.09 mcg./g. In unstimulated controls the slight average increase to 3.29 mcg./g. after 30 min., as well as a marginal decrease to 2.63 mcg./g. at 60 min., was not significant. Stimulation of the peripheral vagi decreased serotonin concentration to 2.48 and 2.73 mcg./g. at 30 and 60 min., respectively. The 30-min. concentration was significantly different from both prestimulation and 30-min. controls, but at 60 min. this change was not significant. Central vagal stimulation decreased duodenal serotonin to 1.97 mcg./g. at 30 min. and 2.29 mcg./g. at 60 min. Both concentrations were significantly different with respect to prestimulation control, but only the 30-min. sample was statistically different from its respective control.

## DISCUSSION

Pentilla (6) demonstrated a significant correlation between the number of EC cells and duodenal serotonin concentration in the mouse, rat, guinea pig, rabbit, sheep, pig, cow, and horse. The regression correlation coefficient of his study was determined on interspecies data, but the duodenal serotonin data from any one species generally did not appear to reflect this correlation. However, there was a modest correlation of EC and serotonin concentration in the rat duodenum during the first 2 months of life (6), and a strong correlation for developing chicken embryonic duodenum (7). Even more significantly, Pentilla (8) reported serotonin concentra-

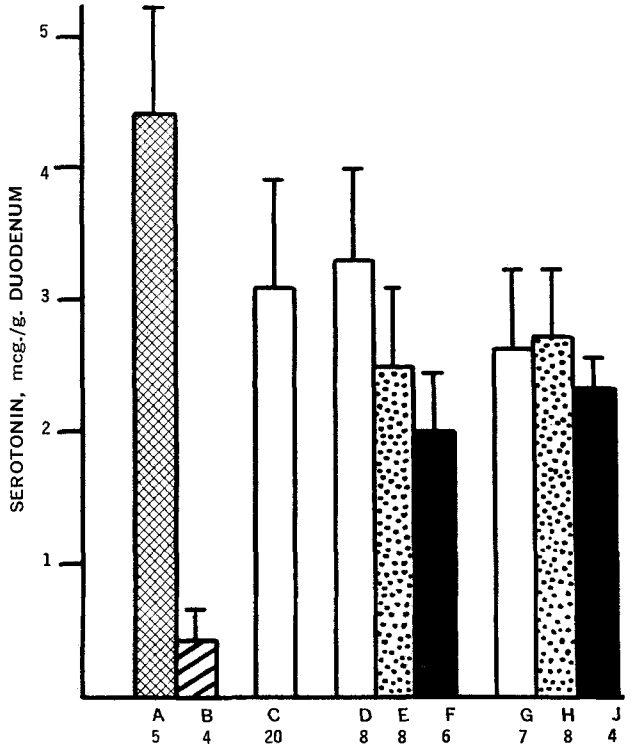


**Figure 1**—*Serotonin concentrations in guinea pig duodenum. Bars A and B: unanesthetized; Bar B: reserpinized; Bars C–J: anesthetized; Bar C: immediately after anesthetization; Bars D–F: 30 min. after stimulation; D: control; E: peripheral stimulated; F: central stimulated; Bars G–J: 60 min. after stimulation; G: control; H: peripheral stimulated; and J: central stimulated. Each bar represents the mean value plus standard deviation. The numbers under the bars represent the number of animals in each group.*

tions and cell counts throughout the alimentary tract in the guinea pig and the correlation was highly significant.

Linear correlation of the cell count with serotonin content must be restricted, however, to normal animals, since in reserpine-treated guinea pigs no such correlation exists (6). Reserpine administration reduces the staining response to silver salts (9), suggesting release of reactive substances from the EC cells. Pentilla observed about a 30–57% decrease in EC counted, whereas serotonin concomitantly decreased by approximately 90%. In the present study, reserpine produced a 90% decrease in serotonin concentration. In another report, a 90% reduction in cells counted was associated with reserpine administration (10).

The serotonin concentrations in guinea pig duodenum determined in this study are in good agreement with Erspamer (11), Nobili (12), and Hagmuller *et al.* (13). Pentilla (6, 8) reported much higher concentrations, which may have been due partly to the use of female guinea pigs in his study, since it is known that they contain a greater number of EC (14). Reserpine administration in the present study was used to demonstrate that the serotonin assay was capable of detecting greater changes in tissue serotonin.

Stimulation of the peripheral vagi produced approximately a 75% reduction in EC counted in the guinea pig duodenum (4), whereas similar stimulation resulted in only an 18% decrease in duodenal serotonin concentration. Central vagal stimulation produced a 46% decrease in cells counted (10) and a 36% decrease in tissue serotonin. A lack of correlation between cells counted and serotonin concentration should be expected. Cell counting is an all-or-none experience, *i.e.*, a cell is counted only if it contains sufficient stain to recognize the granules by which they are currently identified. Once a cell contains an unknown threshold concentration of stainable material, the addition of more stainable substrate would not alter the count. Conversely, cells may contain a considerable concentration of serotonin and yet, if subthreshold or in an unstainable form, may be insufficient to produce visual detection of granules. It should also be emphasized that tissue serotonin assay does not distinguish between serotonin granules within EC and released amines. Nonetheless, the observed reduction in granulation by peripheral vagal stimulation would at least be expected to be associated with a decrease in serotonin concentration, which is what the authors observed. Extension of the sampling time 30 min. beyond the end of stimulation to permit degradation or removal of released serotonin did not bring about any further decreases in serotonin.

Other investigators claimed demonstration of significant release of serotonin into the venous circulation of the vascularly perfused dog small intestine by administration of catechol amines (15) or by stimulating arterial sympathetic nerves. However, they failed to consider several factors. First, both parasympathetic and sympathetic nerves are grossly indistinguishable on the surface of the intestinal arteries; since no evidence was offered to support the statement that only sympathetic nerves were stimulated, it may be assumed that both types of fibers were involved in their study. Secondly, they reported changes in serotonin concentration in the effluent of the perfused intestine without indicating any flow data. Catechol amines and nerve stimulation would be expected to alter greatly the flow through the intestine; accordingly, without flow information, net changes in serotonin release are unknown. Therefore, the data they presented do not seem to demonstrate their conclusions unequivocally. On the other hand, this report is the first to demonstrate a reduction in intestinal tissue concentration of serotonin in response to autonomic nerve stimulation. The types of nerve fibers involved in this response are considered elsewhere (10).

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## ACKNOWLEDGMENTS AND ADDRESSES

Received April 27, 1970, from the *Schools of Dentistry and Pharmacy, Temple University, Philadelphia, PA 19140*

Accepted for publication September 21, 1970.

This investigation was supported by Grant AM-11372-02 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

The authors thank Mrs. Eileen Miller for her assistance in the fluorometric determination of serotonin.