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EFFECTS OF RESERPINE AND COLCHICINE ON ENTEROCHROMAFFIN CELLS*

BERNARD H. MARKS and ROBERT W. SORGEN

Department of Physiological Chemistry and Pharmacology,
The Ohio State University College of Medicine

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Abstract—Enterochromaffin cells can be distinguished in preparations of rabbit duodenal mucosa stained supravitally with neutral red. In reserpinized rabbits, these cells show a loss of granulation in from 4 to 6 hr, corresponding to that previously observed in studies of fixed and stained sections.

Colchicine-treated rabbits also demonstrate a delayed degranulation of enterochromaffin cells, both by the supravital and by the stained section methods. This may have some bearing on the hyperperistalsis known to be produced by colchicine.

STUDIES of the functional activity and pharmacological responses of the enterochromaffin cell system of the gastroenteric tract are of considerable potential interest. Not only may these cells have a role in regulating peristalsis,[1] but possibly other enteric activities as well, through their synthesis and release of amines, notably 5-hydroxytryptamine (5-HT).[2] Studies of the activity of this cell, occurring singly as they do buried between enteric epithelial cells, have been restricted to methods involving assay of 5-HT activity released from mucosa[3] or counting of enterochromaffin cells in strained sections of gut.[2, 4] These procedures either produce results in terms of statistical averages of cellular activity, or afford indirect estimates of changes in these cells.

In this investigation, the functional response of enterochromaffin cells has been examined directly by observing the disappearance of granules, presumably the repository of 5-HT in these cells. The enterochromaffin cells, which have been located and examined in supravital-stained preparations, have been shown to respond to a 5-HT-releasing drug with the expected loss of staining characteristics.

MATERIAL AND METHODS

All experiments were carried out on young male albino rabbits, weight approximately 3 kg. Animals were injected intravenously with 5 mg of reserpine per kg or with 2 mg of colchicine per kg. Saline-injected controls, sacrificed at the same times as the treated animals, were used.

Segments of duodenum approximately 2 cm long were removed and rinsed gently in saline. The segment was cut lengthwise and spread open upon a glass plate with the mucosa upward. The mucosa was separated from the rest of the strip by scraping

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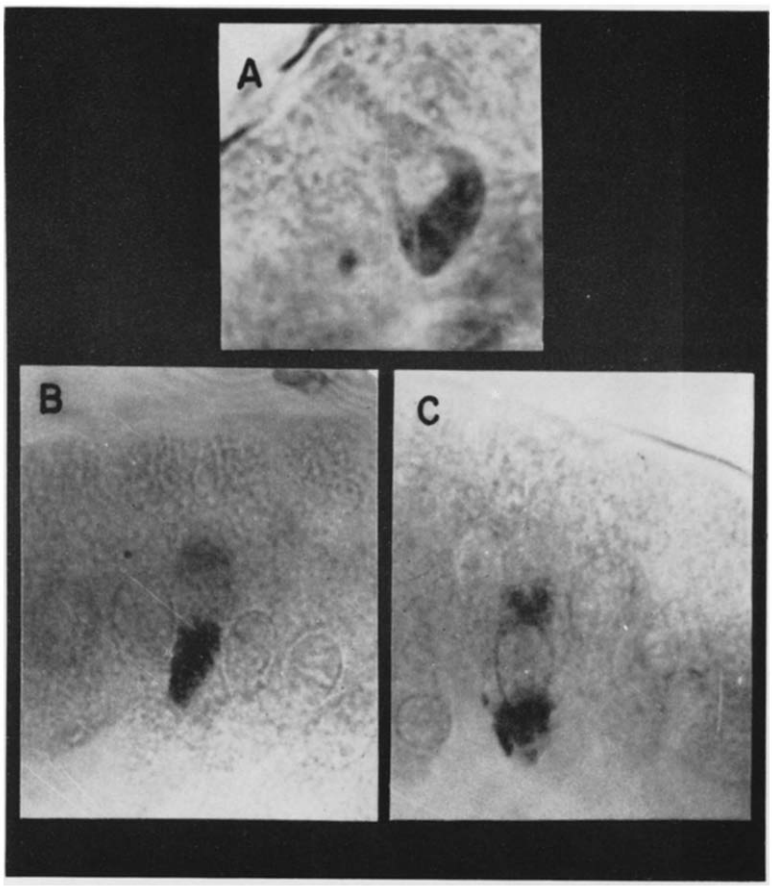

FIG. 1. Supravital preparations of normal rabbit enterochromaffin cells. A. Characteristic flask shape. × 454. B, C. Characteristic distribution of granules. × 360.

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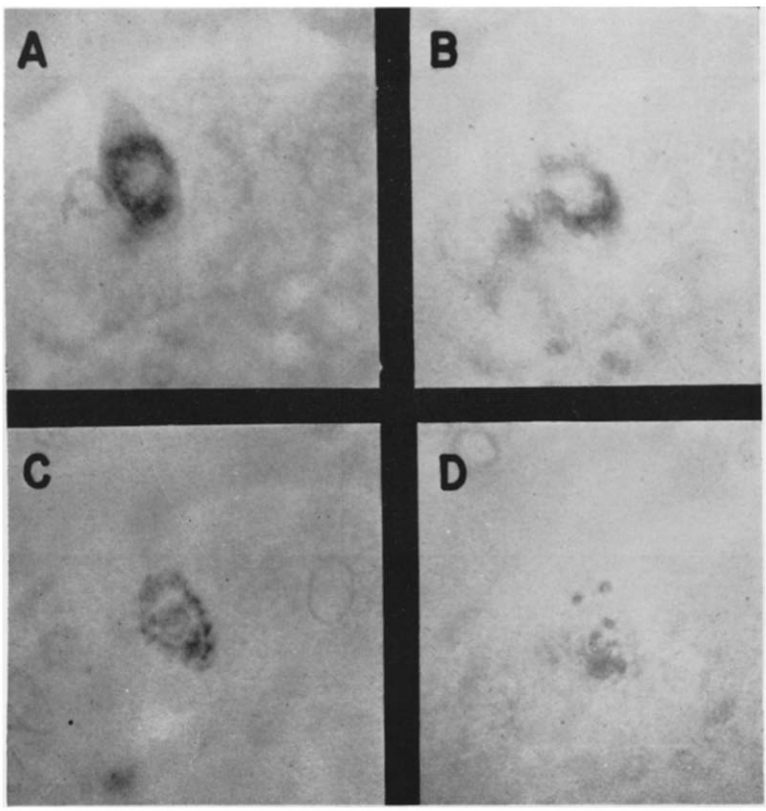

FIG. 2. Supravital preparation of a rabbit enterochromaffin cell after administration of 5 mg of reserpine per kg. A, 3 hr. B, 4 hr. C, 5 hr. D, 6 hr. × 873.

it off, using as a pusher the narrow side of a glass microscope slide, pressed firmly against the tissue at an angle of about 30°. Often, the mucosa could be removed in layers, the first by a gentle scraping, the second by a more forceful one. Under these conditions there were usually more enterochromaffin cells found in the second layer than in the first, in conformity with greater occurrence of these cells in the deeper portions of the mucosal folds noted in our silver-stained preparations.[2]

Small masses of the duodenal mucosa scrapings were transferred to watch glasses and covered with a 1% solution of neutral red in 0·9% saline. These were rotated for from 30 to 60 sec, then the excess dye was poured off and the stained tissue in the watch glass was rinsed with several changes of Hank's balanced salt solution,[5] buffered at pH 7·5, until no more dye was freely removed from the tissue. With dissecting needles, a small mass of this stained tissue, approximately 1 mm^3, was transferred to a drop of buffered balanced salt solution in the center of a circle of petrolatum on a glass microscope slide. The tissue was gently teased apart with the needles and attempts were made to orient the tissue masses into sheets or strands. A cover slip was then placed on top of the preparation and sealed to the circle of petrolatum, care being taken to determine that the seal was intact completely around the circumference of the tissue spread.

Microscopic examination at magnification × 100 revealed enterochromaffin cells in the mucosal scrapings. Individual cells were followed for prolonged periods at this and at higher magnifications. It was possible to examine these cells under phase contrast as well as bright field optical conditions. Photographs of these cells at appropriate intervals recorded the changes in them produced by the actions of drugs.

For comparison with the above supravital staining method, preparation of microscopic sections and staining of the enterochromaffin cells was performed according to methods reported previously.[2] Estimation of the magnitude of granulation in stained sections was performed by counting and weighting the cells in ten high-power fields in each of four sections and calculating a weighted granulation index according to the Hartrofts.[6] The significance of the difference between the means of the weighted indices of the experimental and corresponding control sections was determined by the *t*-test.

RESULTS

In scrapings of duodenal mucosa stained with neutral red, one may observe microscopically a small area of tissue with its component cells arranged as they occur normally *in vivo*. Two kinds of cells in these preparations contain granules stained with neutral red. One of them is not closely associated with the epithelial cells of the mucous membrane, is round, and its granules are extremely dense and so densely packed as to obscure the nucleus of the cell. These we identify as mast cells; although not very numerous in the rabbit duodenum, they do occur regularly.

The other cell, with granules stained red under these conditions, contains fewer and smaller granules than the mast cell, and the granules are predominantly in one end of the cell, the nucleus being seen near the middle. When one examines a piece of tissue properly oriented, these cells are seen to be between the epithelial cells of the mucosa, and in the same layer with them. A typical cell of this species is flask-shaped, with the wide end, containing the granules, toward the basement membrane of the epithelium. The narrow neck of the cell, free of granules, extends to the striated

border of the epithelium. The nucleus is characteristically seen at the shoulder of this cell (Fig. 1). These cells appear to fit the requirements for identification as enterochromaffin cells. Particularly, they are consistent with the electron microscopic observations of Taylor and Hayes[7] on the structure of this cell.

As a test for this method of examining enterochromaffin cells, we can study them under conditions which are thought to cause degranulation, as revealed by the older methods of examining stained sections. One such condition is the administration of reserpine, 5 mg/kg.[2] A series of photographs of one enterochromaffin cell from the duodenum of a reserpinized rabbit is shown in Fig. 2. It can be seen that loss of vital

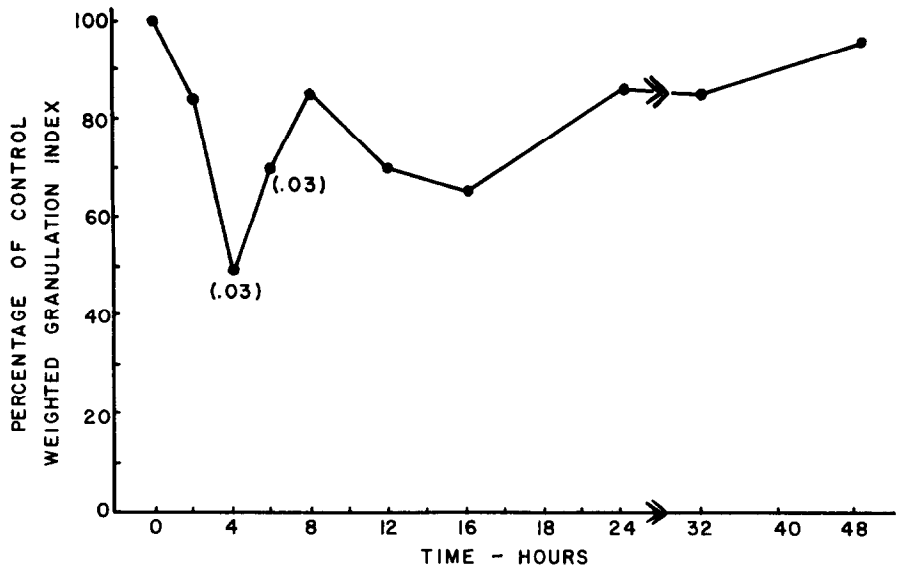

FIG. 3. Change in weighted granulation indices of rabbit duodenum sections after the intravenous administration of 2 mg of colchicine per kg. Figures in parentheses are the *P*-values for observations that show significant differences from the controls.

staining of this cell began in from 3 to 4 hr and was virtually complete in 6 hr. With saline controls, no changes such as those recorded here were seen in this period of time. Although some enterochromaffin cells in each preparation were not degranulated, the vast majority showed a loss of granule-staining in response to reserpine. Mast cells were still recognizable at a time when the specific destaining of enterochromaffin cells had already occurred.

As a further test of this method of examining enterochromaffin cells, we studied them both supravitally and in stained sections following the administration of colchicine. Fig. 3 gives the results of the quantitative evaluation of cellular granulation in rabbits sacrificed at various time intervals following the intravenous administration of 2 mg of colchicine per kg. Significant degranulation ($P = 0{\cdot}03$) occurred at 4 and 6 hr after administration; however, the difference between the treated and control animals had essentially disappeared after 24 hr. Likewise, in supravital preparations of duodenal mucosa made by the neutral red method 2 hr after the

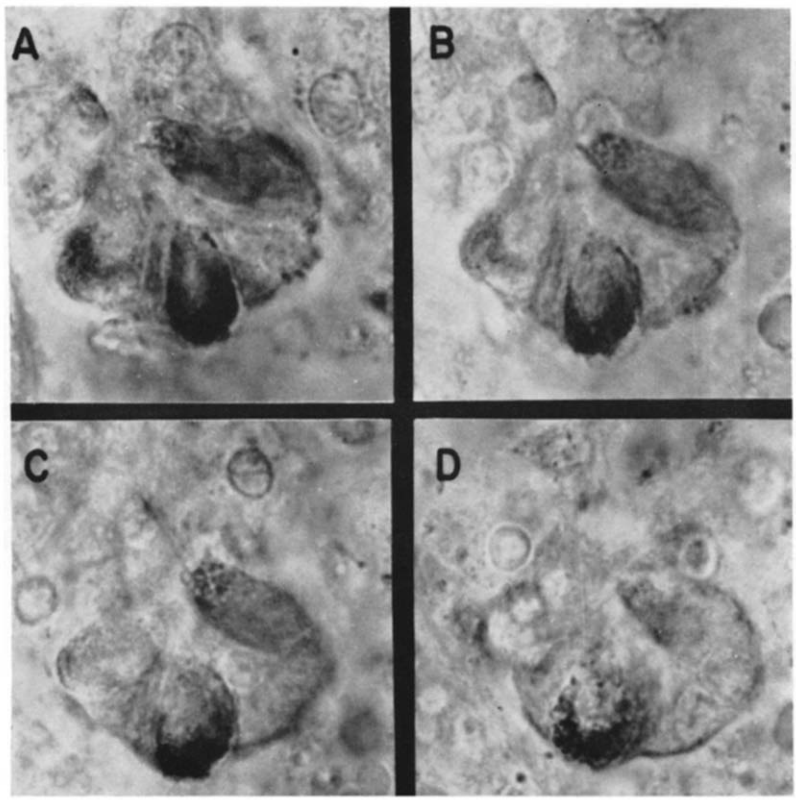

FIG. 4. Supravital preparation of a rabbit enterochromaffin cell after the intravenous administration of 2 mg of colchicine per kg: A, 3 hr; B, 4 hr; C, 5 hr; D, 6 hr. × 360.

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intravenous administration of 2 mg of colchicine per kg, evidence of cellular change was observed in a number of instances in the succeeding few hours (Fig. 4).

DISCUSSION

The use of supravital-stained preparations of duodenal mucosa directly demonstrates loss of staining of enterochromaffin cell granules in response to reserpine. The delay required for this occurrence and the delay generally observed in producing hyperperistalsis are parallel and indicate the functional role of released serotonin in producing this response.

Though one cannot readily obtain quantitative data from the examination of a few single cells, such direct observation is a rapid method for screening drugs for possible effects on cells of this type.

Colchicine (or colchicum) has been known since antiquity to produce a characteristic delayed hyperperistalsis when administered in large doses. Fuehner and Rehbein[8] showed long ago that this drug had no direct action on strips of intestinal muscle. Observations reported here indicate that, like reserpine, colchicine may induce a delayed release of the contents of enterochromaffin granules. This is shown both by the study of fixed and stained sections and by the examination of supravital-stained enterochromaffin cells. This finding indicates that serotonin may play a functional role in producing the hyperperistalsis associated with colchicine administration.

SUMMARY

Supravital staining with neutral red is shown to distinguish enterchromaffin cells. These cells show a destaining response to reserpine. Both in stained sections and by supravital staining, colchicine is also shown to induce delayed loss of granulation of enterochromaffin cells.

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