EFFECT OF RESERPINE ON THE ENTEROCHROMAFFIN CELLS OF THE SMALL INTESTINE

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Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 2, pp. 78-81, February, 1961
Original article submitted March 1, 1960

At the end of the last century, N. K. Kul'chitskii (1897) described the enterochromaffin cells of the mucous membrane of the digestive tract. These cells have recently attracted the particular attention of researchers both here and abroad. Not long ago, Erspamer and Asero [6] isolated serotonin (5-hydroxytryptamine), which influences the vascular tonus [5], from a carcinoid tumor of these cells.

Numerous investigations have recently been published concerned with serotonin's effect on various organs and tissues, its distribution in the different systems of the organism and the search for serotonin antagonists. The data indicating serotonin's role in increasing arterial blood pressure are especially important [5]. The histochemical investigations of Benditt and Wonj [4] established that enterochromaffin cells contain about 1% serotonin. Under the influence of reserpine [7], serotonin disappears from the enterochromaffin cells [10] and is decomposed by monoamine oxidase. In their article [3], A. I. Yakovleva and N. G. Shakhnazorova showed that, depending on the condition of the animals (starved and not starved) and on the method of reserpine administration, reserpine caused the argyophil granules in enterochromaffin cells to disappear. These works investigated the effect of a single administration of reserpine in large doses on the morphology of enterochromaffin cells. We were unable to find in the literature available information on the effect of reserpine, systematically administered for a prolonged period, on the enterochromaffin cells. Nor has reserpine's effect on the number of enterochromaffin cells yet been investigated.

We therefore decided to conduct a special study under conditions of a chronic experiment to investigate the effect of repeated administrations of reserpine in comparatively small doses on the enterochromaffin apparatus of the small intestine, specifically, that of the duodenal mucous membrane. This portion of the intestine was selected for study because, in guinea pigs, it is known to contain the greatest number of enterochromaffin cells and the extremely receptor-rich zone of the small intestine; here the most important digestive juices are secreted, and the reflexes inducing increase of arterial pressure during alimentary stimulation originate from here.

EXPERIMENTAL METHODS

The experiments were performed on eight guinea pigs. The experimental animals were divided into four groups. The guinea pigs of the first, or control, group did not receive reserpine. Daily peroral administrations of reserpine were given to the three experimental groups, in a dose of 0.05 mg for the second group, 0.125 mg for the third group and 0.25 mg for the fourth group. After receiving reserpine for a month, the animals were decapitated, and various parts of the duodenum were taken for histological examination. The material was fixed with Lison's mixture and with a 10% formalin solution. The paraffin blocks were cut into sections 4-5 microns thick. The acid diazo reaction and Held's hematoxylin stain were used to demonstrate the enterochromaffin cells.

EXPERIMENTAL RESULTS

Considerable numbers of enterochromaffin cells were found in the control animals along the whole length of the duodenum. These cells were arranged singly or in groups and usually located in the epithelial lining of the crypts, occasionally in the substance of the Brunner's glands and also in the epithelial sheath of the villi (Fig. 1). The bodies of the cells were irregularly shaped. In the protoplasm of these cells, the acid diazo reaction demonstrated profuse, reddish-brown granulation. In some of the cells, the granulation was more or less evenly distributed. Cells with basally situated granulation were, however, observed (Fig. 2). Most of the enterochromaffin cells were found on the bottom of the crypts or on adjacent areas. Much more rarely, enterochromaffin cells were found in the substance of the epithelial sheath covering the lateral surfaces of the villi. In these cells, the granulation was somewhat finer, less abundant and basally situated.

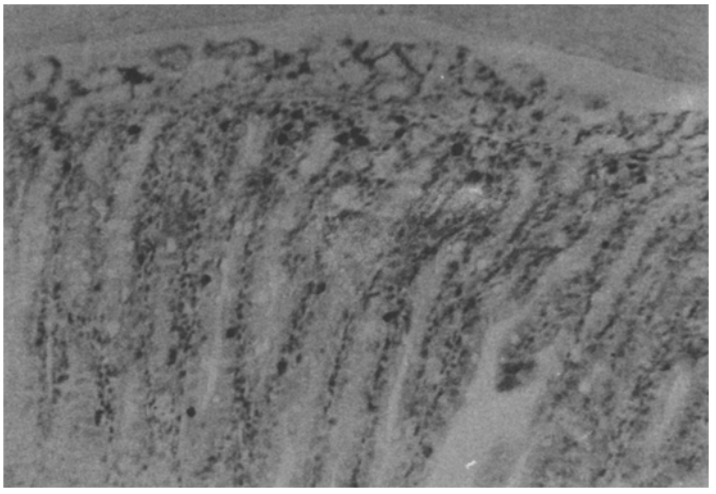

Fig. 1. Duodenal mucous membrane of control guinea pig. Many enterochromaffin cells (the dark cells) are apparent on the bottom of the intestinal crypts, as well as in the epithelial sheath of the main villi. Acid diazo reaction with hematoxylin afterstain. Magnification 200 ×.

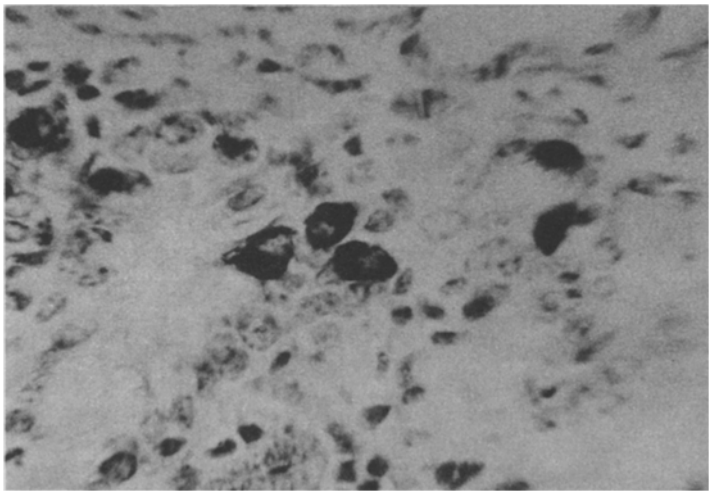

Fig. 2. Duodenal mucous membrane of the same animal. Profuse granulation is seen filling the apical and basal portions of the cells. Acid diazo reaction with hematoxylin afterstain. Magnification 1000 ×.

TABLE 1

Change in Enterochromaffin Cells Effected by Reserpine

Reserpine dosage	Total number of enterochromaffin cells			
	M	M	t	D
Control (reserpine not administered)	171	23.61	–	–
0.05 mg per day	127	16.8	1.55	0.05
0.125 mg per day	96	9.2	3.0	0.01
0.25 mg per day	103	8.7	2.3	0.03

TABLE 2

Effect of Reserpine on Granulation Content of Enterochromaffin Cells

Reserpine dosage	Enterochromaffin cells with large number of granules		Enterochromaffin cells with small number of granules	
	number	%	number	%
Control (reserpine not administered)	107	62.5	64	37.5
0.05 mg per day	56	44.1	71	55.9
0.125 mg per day	33	34.4	63	65.6
0.25 mg per day	40	38.9	63	61.1

In the experimental animals, both the total number of enterochromaffin cells (Fig. 3) and the granulation content of the cells (Fig. 4) were found to be reduced under the influence of reserpine. We tabulated the number of enterochromaffin cells, dividing them into two groups: 1) the cells containing a large number of granules; 2) the cells containing a small number of granules. We computed the arithmetic mean (M), the square deviation (δ), the standard error (t) and the authenticity of the difference (D) between the different groups according to the Student-Fisher index.

Table 1 gives data showing the changes in the total number of enterochromaffin cells affected by different dosages of reserpine.

As Table 1 shows, the average number of enterochromaffin cells per 100 crypts was 171 in the control animals. In the animals which had received 0.05 mg reserpine daily for a month, the average number of enterochromaffin cells was reduced to 127 per 100 crypts. The decrease in the number of enterochromaffin cells observed after the administration of reserpine in these doses was statistically unreliable and did not exceed the control ($t = 1.55$, $D < 0.05$).

The changes were considerably more pronounced in the animals which had received 0.125 mg reserpine daily for a month than in the preceding experimental variant. In this case, the number of enterochromaffin cells decreased to 96 per 100 crypts. This decrease in the number of cells was found to be statistically authentic ($t = 3.0$, $D < 0.01$) as compared with the control. The number of enterochromaffin cells was also reduced in the animals which had received the 0.25 mg daily dose of reserpine (103 cells per 100 crypts). The difference between the control and the experiment was found to be statistically authentic in this case also ($t = 2.3$, $D < 0.03$).

Reserpine induced a change in the intimate structure of the cells as well as the decrease in their total number. The number of granules in the cells decreased, and the main mass of granulation moved into the apical part of the cell (see Fig. 1). The zone located under the nucleus of these cells was usually free of granules definable by the acid diazo reaction or Held's hematoxylin. The larger doses of reserpine caused the enterochromaffin cells to lose their definite outlines and to become smaller. Single tiny granules could be barely seen against the clear background ot the protoplasm. A typical effect of reserpine in these cases was a marked change in the correlation between the enterochromaffin cells which contained numerous granules in the protoplasm and those with a low granule content. These data are given in Table 2.

As Table 2 shows, 62.5% of the enterochromaffin cells had a high granule content and 37.5% contained a small number of granules (the ratio of the cells with a large number of granules to those with a small number constituted 2:1).

After the administration of reserpine in a daily dose of 0.05 mg, 44.1% of the cells contained a large number of granules, 55.9%, a small number, giving a ratio of 4:5.

Therefore, reserpine caused a marked decrease in the number of enterochromaffin cells with a high granulation content. This change was even more marked when reserpine had been administered in a daily dose of

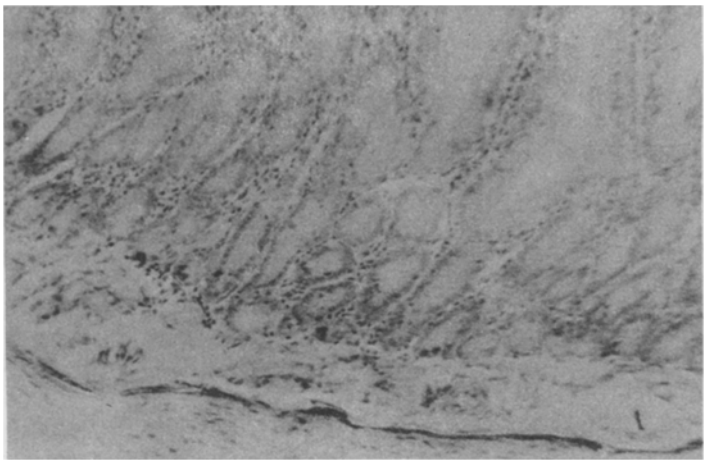

Fig. 3. Duodenal mucous membrane of guinea pig given reserpine in a dose of 0.25 mg a day. Sharp decrease in the content of enterochromaffin cells in the intestinal mucous membrane. Only 4-6 enterochromaffin cells evident in the entire field of vision. Most of the crypts devoid of enterochromaffin cells. Acid diazo reaction with hematoxylin afterstain. Magnification 200 ×.

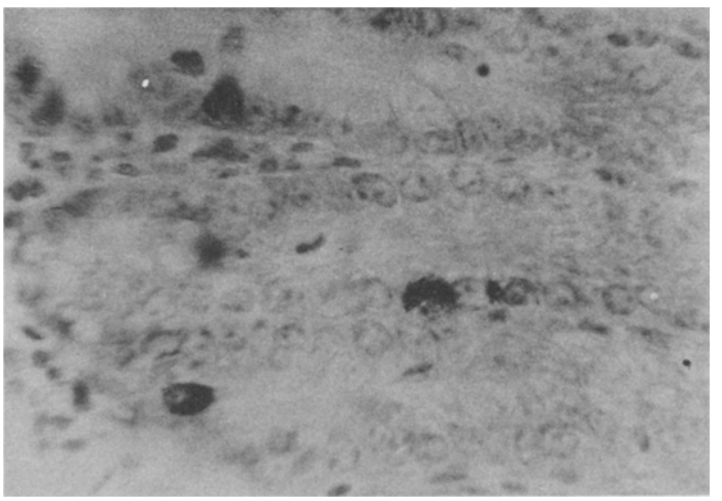

Fig. 4. Duodenal mucous membrane of the same animal. Reduced granulation content in enterochromaffin cells. Granulation has shifted to apical portion of cell. Acid diazo reaction with hematoxylin afterstain. Magnification 1000 ×.

0.125 mg. With this dose, the number of cells with a large number of granules decreased to 34.4%, and the number of cells containing few granules increased to 65.6%. Their ratio, then, was 1:2. Finally, when reserpine was administered in doses of 0.25 mg a day, the cells with a large number of granules comprised 38.9%, while 61.1% contained a small number, i.e., the decrease in the granulation content of the cells is clear.

The observations we conducted, therefore, show that the administration of reserpine to the guinea pig organism for a month decreased the total number of enterochromaffin cells in the duodenum and either diminished the formation of granules in the protoplasm of enterochromaffin cells or caused the complete disappearance of these granules.

Since enterochromaffin cells produce serotonin, which definitely participates in the rise of arterial pressure, the decrease in arterial pressure which attends the prolonged administration of reserpine, both under normal conditions and under conditions of hypertonia, is probably due chiefly to the effect of reserpine on the enterochromaffin cells.

In our earlier investigations [1], we established that reserpine reduces both increased and normal blood pressure in experimental animals. The effect of reserpine perorally administered is only apparent after 3-5 days. The long duration of this preparation's latent effect is evidently due to its action on the systems by means of which a certain blood pressure level is maintained, possibly to its action on the enterochromaffin cells.

SUMMARY

To investigate the mechanism of the reserpine effect on the blood pressure an experiment was done on 8 guinea pigs with a corresponding control. Experimental animals were given reserpine in the doses of 0.05 mg, 0.125 mg and 0.25 mg. No reserpine was administered to control animals.

In a month after the reserpine administration was started the animals were sacrified and various portions of their duodenum were subjected to histological examination. The total number of enterochromaffin cells in the duodenum was found to be reduced; the formation of the granules in the protoplasm of chromaffin cells was diminished or these granules were totally lost.

A suggestion is made that the drop of arterial pressure occurred as a result of reserpine action upon the enterochromaffin cells of the small intestine, which produce serotonin.

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