USE OF THE MICROVIDEOMAT TELEVISION ANALYZER TO ASSESS THE FUNCTIONAL STATE OF THE ENTERO-CHROMAFFIN CELL POPULATION OF THE RAT DUODENUM

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Many recent investigations [1, 5, 6, 10] have shown that local hormones of the gastrointestinal tract play an important role in the system of neurohumoral regulation of digestive functions. Accordingly information on disturbance of the state of the entering system in various pathological processes such as duodenal and gastric ulcer, the dumping syndrome, the Zollinger–Ellison syndrome, the malabsorption syndrome, and so on, is of great interest [4, 7, 12]. However, despite many investigations, some of them morphological, on this subject no information could be found in the literature on morphological methods for quantitative analysis of the functional state of the endocrine cell population. Yet such an assessment is essential if the mechanisms of disturbance of their secretory activity under experimental and pathological conditions are to be elucidated.

An attempt was accordingly made to make a quantitative study of the functional state of the enterochromaffin cell population of the duodenum by photometric estimation of the result of the Masson–Hamperl reaction, which reliably detects serotonin in cells of EC type [8, 11].

EXPERIMENTAL METHOD

Two groups of noninbred male albino rats, six animals in a group, weighing 150-180 g were used; the rats were killed in pairs (experiment–control) after starvation for 24 h and 3 h after being fed once with a standard quantity of mixed grain. Pieces of duodenum 1 cm long from the region of the pyloric sphincter were fixed for 24 h in 10% formalin solution buffered by Lilly's method to pH 7.0. The material was embedded in combined blocks to obtain control and experimental sections of equal thickness.

Measurements were made on a Microvideomat television image analyzer (from Opton, West Germany) using the Ph-nZ program [9]. Cytophotometric analysis enabled two phases of the histochemically detected secretion product to be distinguished in a single cell: an optically dense phase, located in the basal part, and a light phase, distributed mainly in the apical part of the cell. The following parameters were recorded: area, mean and total absorption of the secretion product for the whole cell, and also for each phase isolated separately. Altogether 365 cells in the control and 335 cells in the experiment were measured.

The results were processed by the Wang-720C computer and presented as histograms. To make the results of measurements more comparable, control histograms were normalized on their own mean, and the experimental histograms on the mean of the corresponding control.

EXPERIMENTAL RESULTS

Analysis of the parameters of distribution of values characterizing the state of the cell as a whole shows that under starvation the normal state of the population of EC cells is characterized by unimodal histograms of distribution of the various parameters with a coefficient of variation of 0.35 ± 0.09 and with marked asymmetry (0.45 ± 0.12; Table 1). Comparison of the experimental and control histograms by the L^2 test shows significant changes ($P < 0.001$) in the state of the EC-cell population toward 3 h after feeding. The distribution of the area of the secretion product was distinctly bimodal (Fig. 1), the excess of distribution of the mean ab-

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TABLE 1. Parameters of Distribution of Area (A), Mean (ME), and Total (TE) Absorption of Histochemically Detected Secretory Product of EC Cells after 24-h Starvation (1) and 3 h after Feeding (2)

		A		ME		TE	
		1	2	1	2	1	2
All cells	*Sk*	0,34	0,44	0,49	0,24	0,57	0,26
	Ex	0,04	−0,51	0,10	−0,66	0,07	−0,96
	P	>99,99		>99,99		>99,99	
Light phase	*Sk*	0,41	0,43	−0,44	−0,62	0,50	0,12
	Ex	−0,12	−0,64	0,33	−0,56	−0,03	−1,14
	P	>99,99		>99,999		>99,999	
Dark phase	*Sk*	0,59	0,75	−0,15	−1,02	0,71	0,54
	Ex	0,05	0,18	2,56	2,71	0,08	−0,66
	P	<95		>99,999		>99,95	

Legend. Sk) Coefficient of asymmetry; Ex) excess of distribution; P) level of significance of difference.

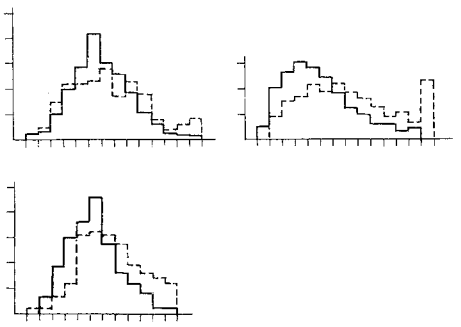

Fig. 1

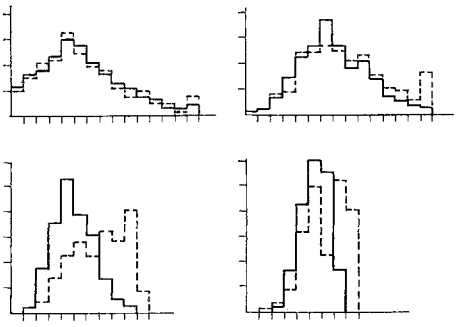

Fig. 2

Fig. 1. Distribution of area (A), mean (ME), and total (TE) absorption of histochemically detected secretion product of rat duodenal EC cells. Continuous line – starvation for 24 h; broken line – 3 h after feeding.

Fig. 2. Distribution of area (A) and mean (ME) absorption of optically dense (I) and light (II) phases of histochemically detected secretion product of EC cells. Legend as in Fig. 1.

sorption was reduced from 0.10 to −0.66, and the total absorption was reduced from 0.07 to −0.96 (Table 1). The proportion of cells with twice the average total absorption increased from 3 to 15% (Fig. 1).

The distribution of area of the light phase of the secretory product also was characterized by a significant reduction in excess and an increase toward 3 h after feeding by 11% of the fraction of cells with an area of substance lying between 1.5 and 2.0 relative units. The histogram of mean absorption of the light phase became bimodal (Fig. 2a, b).

The character of the changes discovered toward 3 h after feeding in the basal part of the cell was significantly different. No changes were observed here in the character of distribution of the area, but the fraction of cells containing secretory product in their basal part with a mean absorption of between 1.25 and 1.45 relative units increased significantly (by 40%) (Fig. 2c, d).

Comparison of the results of quantitative analysis of the state of the EC cell population of the rat duodenum under normal "hungry" conditions and 3 h after feeding led to several suggestions regarding the mechanisms of secretory activity of the enterochromaffin cells. The asymmetrical character of the distributions describing the state of population after starvation for 24 h can be explained, in our opinion, by the fact that in the absence of food intake the population is exposed to the constant action of stimuli of weak intensity inducing a discharge, as a result of which most of the cells periodically expel small portions of secretory product but

do not lose it completely (phenomena of this sort have been described in relation to the exocrine part of the pancreas [3]). It can be tentatively suggested that a reorganization of the population takes place, as a result of which the fraction of cells containing much secretion product is reduced, and this leads to the appearance of asymmetry of the distribution histograms.

In response to the food stimulus some cells of the population perhaps liberate their secretory product completely and thereupon enter on a new secretory cycle, so that 3 h after feeding a considerable quantity of secretion has accumulated (1.5-2 times more than during hunger). This is manifested on the histograms by the appearance of a biomodal curve or a decrease in the excess.

The different character of the changes in the histograms describing the state of the dense and light phases of the secondary product, in the writers' view, reflects the presence of apicobasal differentiation of the enterochromaffin cells. The apical part of the enterochromaffin cell is known to be a zone of synthesis of the secretory product, whereas the basal zone performs a storage function [2, 8]. An increase in the area of the light phase of the secretory product in the apical part of the EC cells, responding to the load, may be explained by the appearance of new areas of cytoplasm occupied by secretory products in these cells, i.e., by the resumption of synthetic processes. The increase in the density of the basal pole of the cell with no increase in area of the dense phase evidently reflects the concentration of secretory product in the zone of deposition.

By the method of television photometry it is thus possible to detect and assess quantitatively functional changes in the state of the EC cell population of the duodenum after food intake and the trend of changes taking place in them. It is also possible to demonstrate functional differences between the apical and basal poles of the cell. It can be concluded from the facts described above that the use of this method opens up prospects for a more detailed investigation of the mechanisms of secretory activity of the enterochromaffin cells.

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