BIOGERONTOLOGY

Regulating Effect of Epithalone on Gastric Endocrine Cells in Pinealectomized Rats

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Endocrine cells in the stomach of pinealectomized rats after injection of epithalone (pineal gland peptide) were studied by immunohistochemical tests, morphometry, and image analysis microscopic images. A functional relationship was found between the pineal gland and stomach, which is regulated by peptides produced by the pineal gland.

Key Words: *pinealectomy; stomach; endocrine cells; epithalone*

Pineal gland (PG) is related to other endocrine organs, while pineal hormones regulate various physiological functions [5]. PG secretes two types of bioactive substances: indolamines, best studied of which is melatonin, and peptides. Melatonin plays the key role in the regulation of biological rhythms and exerts various effects on the nervous, endocrine, and immune systems [11]. In the organism melatonin is produced by pinealocytes and extrapineal cells [9,10]. Cells synthesizing melatonin and its main precursor serotonin are abundant in the gastrointestinal tract, which prompted attempts at elucidating the role of pineal melatonin in the regulation of gastrointestinal functions [6,8].

Peptides secreted by PG and their physiological effects are less studied. High activity of low-molecular-weight polypeptides isolated from PG has been demonstrated [1,7]. Epithalamine, polypeptide complex isolated from PG by acetic acid extraction, regulates the functions of many organs and systems [2]. Epithalone, a new synthetic tetrapeptide (Ala-Glu-Asp-Gly) with a higher biological activity was synthesized on the basis of epithalamine amino acid composition [4].

In order to elucidate the regulatory role of PG tetrapeptides in reparation of impaired neuroendocrine relationships, we investigated functional morphology and behavior of gastric endocrine cells in pinealectomized animals injected with epithalone.

MATERIALS AND METHODS

The study was performed on 25 male Wistar rats (130-140 g) kept under standard vivarium conditions at daylight and fed balanced rations during autumn-winter (November—December). Control group consisted of intact rats. In rats of 4 experimental groups pinealectomy (PE) was performed under ether narcosis. A 1-1.5-cm incision was made along the median line of the head. After exposure of the skull roof, a 0.5-cm hole was drilled with a special bore above the site of fusion of the dura mater sinuses. PG was removed through the hole with ophthalmological pincers. The trephanation opening was covered with removed bone fragment and skin incision was sutured with silk. Postoperation survival was 83%. Starting from day 21 postoperation the animals of groups 1 and 2 were daily (for 10 days) subcutaneously injected with isotonic NaCl (0.5 ml), while groups 3 and 4 rats received an

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equivalent volume of epithalone according to the same protocol (total dose 0.5 μg/rat)

The material was collected in the morning (10.00-12.00) at daylight on days 30 and 42 after PE under Nembutal narcosis. Fragments of the stomach pylorus were fixed for 24 h in Bouin's fluid. Paraffin sections (7 μ) were placed onto poly-l-lysin-coated slides (Sigma).

Serotonin-producing enterochromaffine (EC) cells were detected immunohistochemically using murine monoclonal antibodies to serotonin (1:15, DAKO) and avidin-biotin-peroxidase kit for detection of mouse immunoglobulins (Vectastain). Gastrin (G) and somatostatin-producing (D) cells were identified using polyclonal rabbit antibodies to gastrin and somatostatin, respectively (1:100, Novocastra) and biotin-streptavidin-peroxidase kit (ISN).

Endocrine cells in the lower and middle thirds of the gastric glands were counted (per mm^2). Optic density of immunohistochemical staining of structures in the same zones was evaluated in arbitrary units. Measurements were carried out in 20 fields (×40 objective) of 2-5 sections of the organ from each animal. Quantitative studies were performed using an IMSTAR computer image analysis system and Morphostar-2 and Coquant-2 software.

The results were statistically processed using Mann—Whitney's nonparametrical *U* test.

RESULTS

In controls, endocrine cells were detected mainly in the lower and middle thirds of gastric glands and were oval or pyramid-shaped (Fig. 1). The product of immunohistochemical reaction was detected in the cytoplasm as granules of different staining intensity. Visually, the differences between the groups were not clearly seen. Endocrine cells in the pyloric part of the stomach actively reacted to PE. Thirty days after PE the density of EC cells was 25% higher than in the control, which was paralleled by a significant increase in the intensity of immunohistochemical staining (Table 1); the count of G cells decreased by 28% and that of D cells was virtually the same as in the control. The ratio of G to D cells was 4.6:1.0 in controls and 3.0:1.0 in pinealectomized animals. Forty-two days after the beginning of the experiment the number of EC cells decreased by 22% in comparison with the control and by 47% in comparison with the previous term. The ratio of G to D cells decreased to 2.6:1.0 due to accumulation of D cells. Hence, the behavior of all endo-

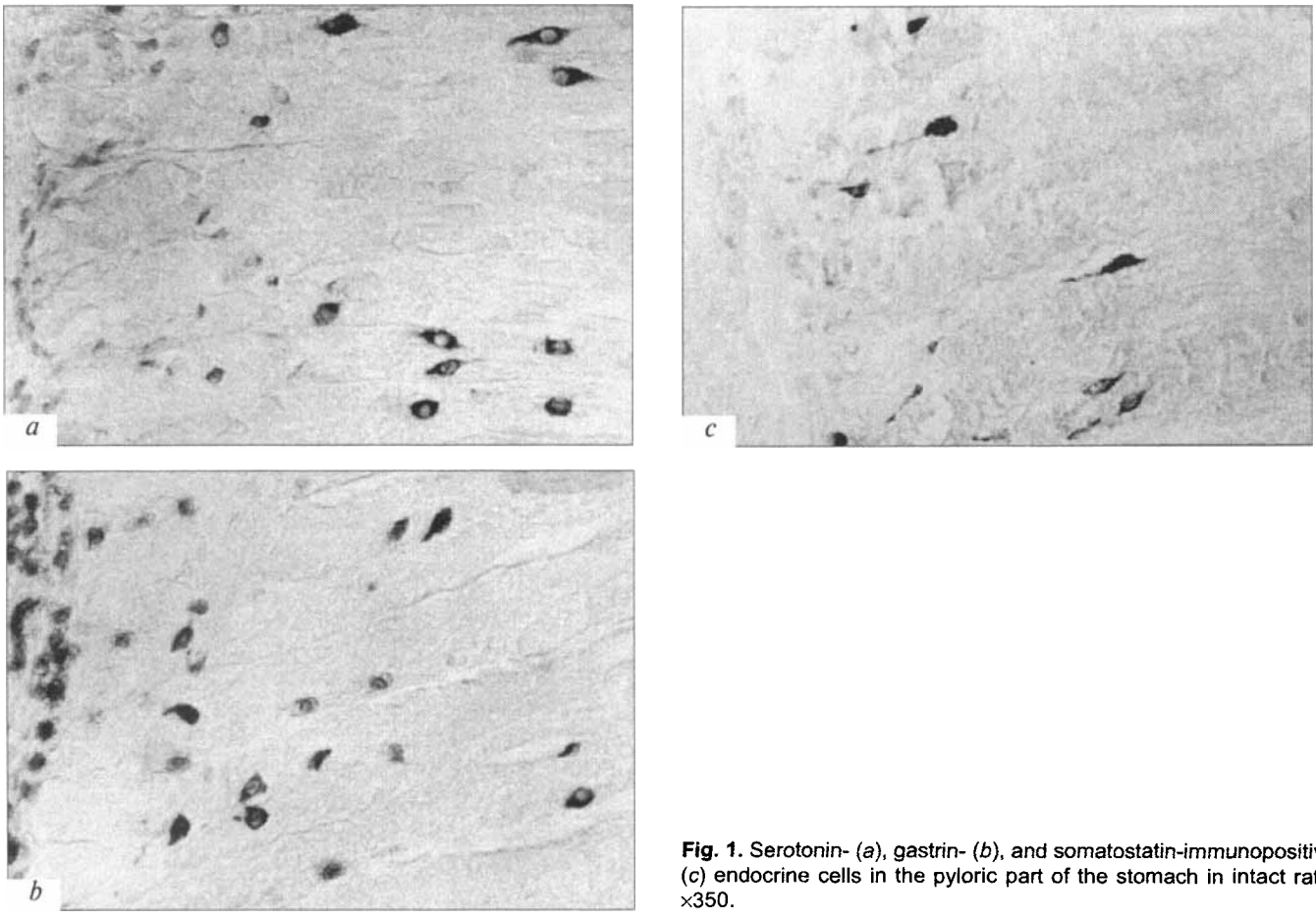

Fig. 1. Serotonin- (*a*), gastrin- (*b*), and somatostatin-immunopositive (*c*) endocrine cells in the pyloric part of the stomach in intact rats, ×350.

TABLE 1. Quantitative Parameters of Endocrine Cells of the Pyloric Part of the Stomach in Rats after PE and Epithalone Treatment (*M±m*)

Cells	Control	Term after PE, days			
		30	30+epithalone	42	42+epithalone
EC cells/mm²	255±10	318±19*	198±10*	263±14	258±21
optical density, arb. units	0.136±0.007	0.168±0.005*	0.156±0.007	0.184±0.005*	0.162±0.010
G cells/mm²	364±23	262±5*	290±11*	323±11	394±34
optical density, arb. units	0.190±0.016	0.220±0.013	0.161±0.002*	0.207±0.006	0.190±0.011
D cells/mm²	79±5	85±6	112±3*	74±7	91±3
optical density, arb. units	0.156±0.007	0.212±0.005*	0.142±0.004	0.154±0.003	0.169±0.007*

Note. *$p<0.05$ *vs.* the control.

crine cells changed after PE and these changes depended on the period postoperation. Presumably, the increase in the count of EC cells 30 days after PE was due to compensatory hyperproduction of melatonin, while the drop of their count after 42 days resulted from increased secretion of this hormone. Serotonin, the main melatonin precursor, also synthesized by EC cells, plays an important role in the realization of the functions of peptide hormones and neuromediators [3], which suggests mediated reaction of G and D cell behavior after PE.

Epithalone essentially modified the behavior of gastric endocrine cells. After 30 days, morphometric parameters of all cells in animals treated with epithalone returned to normal (Table 1). The ratio of G to D cells was the same as in the control (4.4:1.0). However, the optical density of EC and G cells remained elevated in comparison with the control. After 42 days epithalone completely leveled the consequences of PE and all studied parameters of endocrine cell reaction virtually did not differ from the control.

Hence, PE disturbed the relationships between different endocrine cells in the pyloric part of the stomach. Epithalone leveled the consequences of PE, which suggests functional relationships between PG and gastrointestinal tract, determined by peptides produced by PG.

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