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ORIGINAL ARTICLE

Gut Neuroendocrine Cells: Relationship to the Proliferative Activity and Apoptosis of Mucous Epitheliocytes in Aging

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Abstract

OBJECTIVES: Diffuse neuroendocrine system (DNES) cells regulate homeostasis via neurocrine, endocrine and paracrine mechanisms. Extensive effects of peptide hormones and biogenic amines necessitate studying of DNES cell biology in aging. In this connection, the functional morphology of gut neuroendocrine cells (NEC), proliferative activity and apoptosis of mucous epithelial cells in aging have been studied.

MATERIAL AND METHODS: The study was performed on BALB/c-nu mice of 4, 21 and 34 months of age. NEC, proliferative activity and apoptosis of mucous epitheliocytes in stomach and duodenum have been studied by histochemical, immunohistochemical and morphometrical methods.

RESULTS: The total number of NEC shows an increasing trend with advancing age. However, the different types of NEC elicit differential patterns. The total number of epithelial cell nuclei does not show any statistically significant difference during aging. The proliferative activity of mucous epitheliocytes also shows no difference among the three animal groups studied. On the contrary, the apoptotic index increases with advancing age.

CONCLUSIONS: The results demonstrate that various gut NEC show differential behavior with age and their time-courses are dependent on the site of location (stomach or duodenum). The picture seems quite complex to allow a comprehensive interpretation, nonetheless it gives us some useful indications for further investigation. In fact, since the gut does not show evident gross age-related physiological changes, modifications with age in specific biological parameters can suggest the key mechanisms of compensative regulatory processes possibly acting during aging.

Introduction

In 1968–69 Pearse suggested that a specialised, highly organised cell system should exist in organisms, whose main feature was the ability of component cells to produce peptide hormones and biogenic amines. The concept was based on an extensive series of experiments on distinguishing endocrine cells in different organs, identifying endocrine cell-generated products and making a thorough cytochemical and ultrastructural analysis of these cells [1]. Different types of cells widely dispersed throughout the organism have a common ability of absorbing monoamine precursors (5-hydroxytryptophan and L-dihydroxyphenylalanine) and decarboxylating them, thus producing biogenic amines. That ability accounts for the term APUD, an abbreviation of "*Amine Precursor Uptake and Decarboxylation*" used by Pearse to designate the cell series [2].

To date, the APUD series includes over 60 types of cells located in gut, pancreas, urogenital tract, airway epithelium, pineal gland, thyroid gland, adrenals, adenohypophysis and hypothalamus, carotid body, skin, sympathetic ganglia, thymus, placenta and other organs [3]. Meanwhile the appearance of radioimmunological methods and rapid development of immunohistochemistry result in establishing a completely unexpected phenomenon, i.e. the same biogenic amines and peptide hormones were identified in neurons and endocrine cells [4].

The accumulated data did not fit the traditional concepts of hierarchical dependence within two main regulatory systems, viz. nervous and endocrine ones. It became more and more evident that the mechanism of biological regulation should be founded on the coordinated functional interaction between the endocrine system and the central and peripheral nervous system based on the common type of information perception and transmission at subcellular, cellular and tissue levels. Recent data on identification of the same and similar physiologically active substances, acting within the nervous system as neurotransmitters and neurohormones; and, locally or distantly as hormones within the endocrine system, enables both system to be incorporated into the universal diffuse neuroendocrine system – DNES [3,4]. Actually, it should be possible to unite in the organisms the structurally isolated nervous and endocrine systems by means of functional relationships between biogenic amines and regulatory peptides and, to a certain extent, to provide a basis for the concept of integrated functions. Located in practically all organs and producing biologically active substances, the DNES cells are regulators of homeostasis acting via neurocrine, endocrine and paracrine mechanisms [4].

Because, the spectrum of biological effects of peptide hormones and biogenic amines is extremely wide and includes the key aspects of aging mechanisms, i.e. the control of proliferative processes, apoptosis, cell differentiation, cell motility, metabolism of free radicals and many others, it seems to be very important to study the cell biology and functional morphology of DNES in aging.

Material and methods

Experiments have been performed on male euthymic BALB/c-nu mice from INRCA colony, the same strain we previously used in our studies on aging long ago [5]. Three groups of animals of different age have been studied: 4 months, 21 months and 34 months. The term *nu* refers to the recessive nude mutation introduced into inbred BALB/c mice by crossing them with congenitally athymic nude mutants (nu/nu). Our colony, derived from an original stock of BALB/c-nu from Copenhagen, had been of great help in early experiments allowing us to compare "haired euthymic" BALB/c (nu/+) and "athymic nude" BALB/c (nu/nu). The aim of these experiments was to study the mechanisms of thymic influence on non-immunological functions, which often we found to be altered in both young athymic nude and old normal mice. In these early experiments, no differences had been observed in the parameters tested between homozygous BALB/c (+/+) and heterozygous BALB/c (nu/+). With the term BALB/c-nu we address a population made up of both heterozygous and homozygous euthymic mice.

Animals were bred as a close colony and maintained under conventional conditions at 23±2°C ambient temperature and 60±15% relative humidity, with minimum ventilation rate of 10 times/h and 14–10/10–14 h light/dark cycles simulating seasonal variations. They were housed 8 per cage (polycarbonate, open on the top and covered with steel wire lid, 26.7x20.7x14.0 cm deep) and fed with conventional chow (Global diet 2018, Harlan, Italy) and tap water ad libitum. Microbiological monitoring and characterization of animals and environment are routinely performed every three months.

Taking into account the fact that gut is the main organ of the DNES [3] we have studied the functional morphology and cell biology of neuroendocrine cells in stomach pylorus and duodenum.

The following histochemical and immunohistochemical methods were used for identification and study of gut neuroendocrine cells:

- argyrophilic method by Grimelius for total identification of the population of gut neuroendocrine cells;
- argentaffine method according Masson for identification of serotonin-producing EC cells;
- Moser method for identification of apoptotic cells, this method represents a modification of the argyr-

ophilic stain by Gomori in the presence of metenamine (visualization of apoptotic nuclei by using of Moser method is about 95–97%);
- immunohistochemical methods using antibodies to serotonin (BioGenex, 1:100), melatonin (CIDtech Research Inc., 1:100), somatostatin (Novocastra, 1:100), gastrin (Novocastra, 1:100), proliferative cell nuclear antigen (PCNA, Calbiochem, 1:25).

For immunohistochemistry, deparaffined and dehydrated sections were treated with 0,5% H_2O_2 in methanol during 30 min for blockade of endogenous peroxidase. Then sections were washed in 0.1 M phosphate buffered saline (PBS, pH 7.2) and incubated in 10% normal rabbit serum (Dako) for 20 min to reduce non-specific antibody binding. Sections were incubated with the primary antibodies for 2 hr at room temperature, followed by three washes with PBS. For identification of immunohistochemical reaction the avidin-biotin-peroxidase kit (ICN) was used according commercial protocol. For counterstain the Mayer haematoxylin was used.

Morphometrical analysis was performed with the computer image analysis system MORPHOSTAR with the applicative software module COLQUANT (Imstar S.A.). Immunopositive cells and nuclei were counted in 50 randomly selected visual fields, each covering 0.785 mm^2, at x200 original magnification (x20 objective and x10 ocular). At least 5 sections were counted from each sample. The results were expressed per 1 mm^2. The data were analysed with the program STATISTICA^R (Statsoft^R) by using Wylcoxon U-test and Student's t-test as well.

Results

Stomach. The total number of neuroendocrine cells has a tendency to increase correspondingly with the increase of mouse age (604±42 in 4 months, 705±53 in 21 months, 753±53 in 34 months), but the various types of neuroendocrine cells behave differently. So, the number of serotonin/melatonin-producing EC cells was 325±52 in 4 months, 345±49 in 21 months, 389±46 in 34 months. The number of somatostatin-producing D-like cells also increased with age (95±12 in 4 months, 111±17 in 21 months, 126±24 in 34 months). On the contrary, the number of gastrin-producing G cells shows a decreasing trend with advancing age (400±27 in 4 months, 374±34 in 21 months, 327±55 in 34 months). Various data obtained from stomach have been reported in Fig. 1; no statistically significant differences among the three ages studied have been found.

Duodenum. As observed for stomach, the total number of neuroendocrine cells is statistically significantly higher in adult and old mice with respect to young ones (196±14 in 4 months, 311±14 in 21 months, 303±11 in 34 months) (Fig. 2). The number of serotonin/melatonin-producing EC cells also increase with age (186±7 in 4 months, 372±21 in 21 months, 431±22 in 34 months).

No statistically significantly differences with age have been found in both the total number of epithelial cell nuclei (6486±92 in 4 months, 6374±95 in 21 months, 6121±55 in 34 months) and proliferative activity of mucous epitheliocytes (the PCNA index

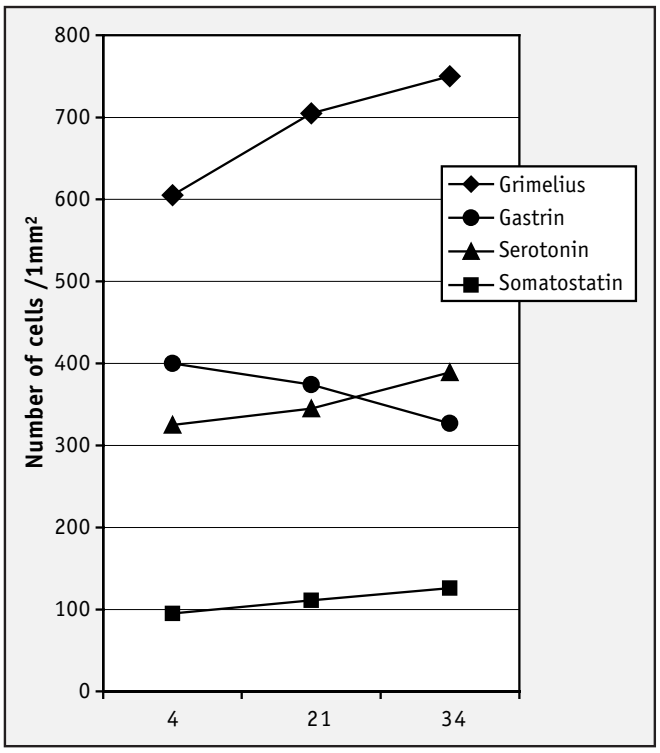

Fig. 1. Neuroendocrine cells in stomach pylorus of mice with different life duration

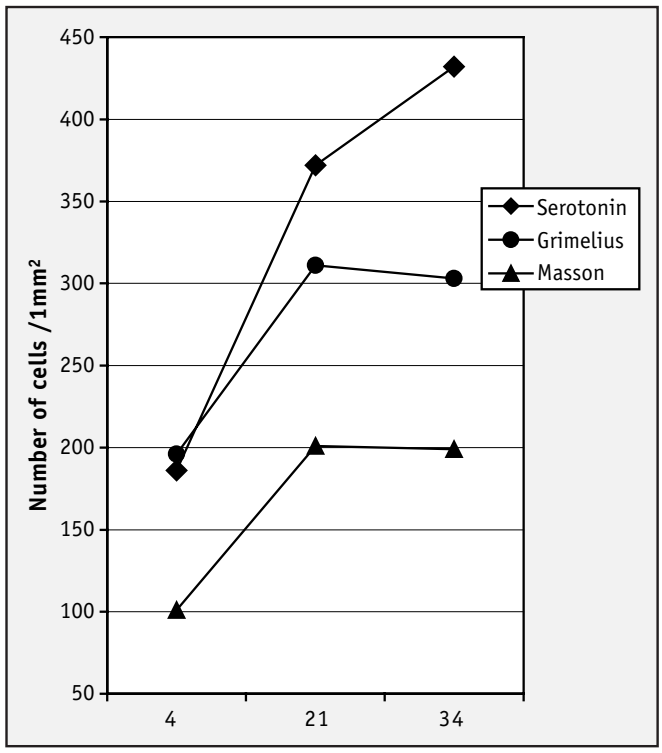

Fig. 2. Duodenum neuroendocrine cells of mice with different life duration

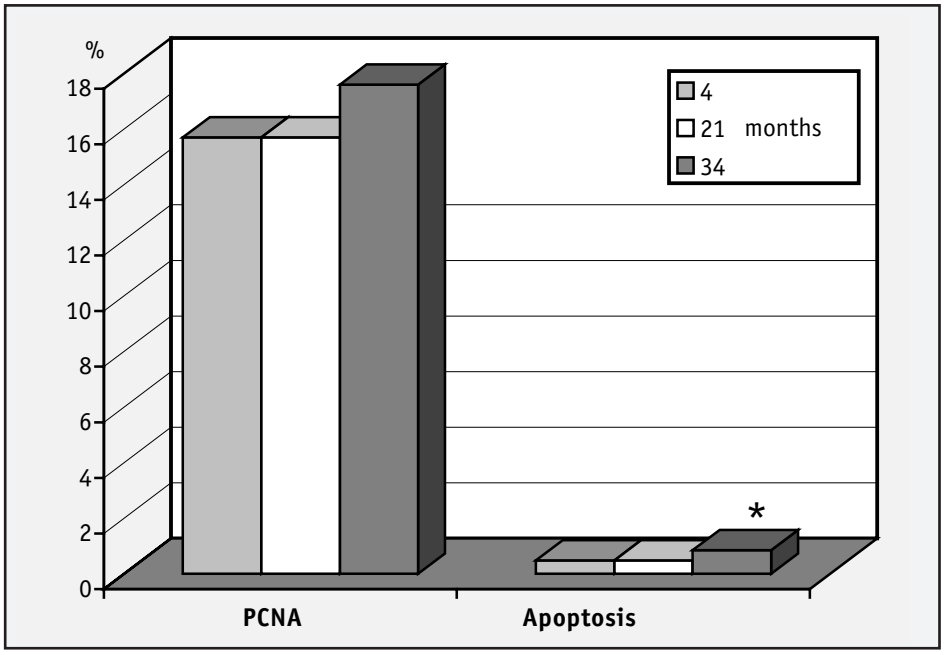

Fig. 3. PCNA and apoptosis indices in duodenum of micewith different life duration.

was 15.7±0.7 in 4 months, 15.7±0.8 in 21 months, 17.6±1.1 in 34 months). On the contrary, the apoptotic index rises with advancing age (0.48±0.06 in 4 months, 0.47±0.06 in 21 months, 0.85±0.09 in 34 months) as shown in Fig. 3.

Discussion

The data presented here demonstrate that stomach and duodenum contain neuroendocrine amine/peptide producing cells that could play an important role in the endogenous mechanisms of aging.

Other researches have studied the functional morphology of several types of gut neuroendocrine cells in animals and humans at different age. In agreement with our data Sandstrom and El-Salhy [6] observed an increase in the number of serotonin immunopositive cells in human duodenum in 20–29 years-old subjects with respect to 1–2 years-old ones; in their study, somatostatin-immunopositive cells also show an increasing trend with increasing age. In other works of the same authors [7, 8] a statistical difference was detected between the different age groups regarding the numbers of gastrin-, somatostatin-, and serotonin-immunoreactive cells. The number of gastrin-immunoreactive cells significantly increased between 1 and 12 months, whereas they became significantly fewer between 12 and 24 months. Somatostatin-immunoreactive cell number increased significantly in 12- and 24-month-old mice, compared with young mice (3 months old). The number of serotonin-immunoreactive cells also increased significantly in 12-month-old mice as compared with young mice. Concerning other gut neuroendocrine cells, the changes of their activity were registered also: the number of cells containing VIP, substance P, galanin and neurotensin all decreased in 12- and 24-old years mice.

Many data have proved that the relations between proliferative activity of cells and their death in apoptotic form play a key role for the speed of aging [9]. Because it is well-known now, that serotonin has a strong anti-proliferative effect and can trigger apoptosis [10], but melatonin on the contrary can prevent apoptosis [11], we attempted to find the correlations between the functional activity of serotonin/melatonin-producing duodenum EC cells and these biological processes.

Our investigations showed an unusual phenomenon: the proliferative activity of epithelial cells does not depend from the activity of EC cells and kept at the same level during all time duration, but the index of apoptosis is increased simultaneously with the prolongation of life duration. These data suggested that the level of cell proliferation is so high, that it can pass ahead of apoptotic elimination, and that it is necessary to carry out further investigations to clarify the interrelations and the role of other regulatory peptides in the endogenous mechanisms of aging.

Thus, the results of this study demonstrate that various gut neuroendocrine cells show differential behavior with age and their time-courses are dependent on the site of location (stomach or duodenum). The picture seems quite complex to allow a comprehensive interpretation, nonetheless it gives us some useful indications for further investigation. In fact, since the gut does not show evident gross age-related physiological changes, modifications with age in specific biological parameters can suggest the key mechanisms of compensative regulatory processes possibly acting during aging.

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