POSTRADIATION DEATH AND RESTORATION OF DUODENAL APUD CELLS IN RATS

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KEY WORDS: APUD system; apudocytes; postradiation death; restoration; secretory granules.

Experimental data on the presence of endocrine cells with general structural and functional properties in nonendocrine organs have led to the creation of the concept of an APUD system [4, 5, 16]. More than 20 types of cells (apudocytes), producing biologically active substances (peptide hormones and biogenic amines) are located in the mucous membrane of the gastrointestinal tract, mainly in the antral portion of the stomach and in the duodenum [14, 17]. This fact has served as a basis for some investigators to regard the intestine as an endocrine organ [9, 12, 13]. Hormones produced by organs of the gastrointestinal tract, with a broad spectrum of biological effects, not only regulate general and digestive homeostasis, but may also evidently make a definite contribution to the pathogenesis of radiation sickness, for symptoms of damage to the gastrointestinal tract arising as a result of exposure of the body to radiation are sufficiently evident, and some investigators distinguish and "intestinal" form of acute radiation sickness [2, 11, 15]. However, the morphological and functional state and the radiosensitivity of the endocrine cells of the gastrointestinal tract during exposure to ionizing radiation remain virtually unstudied. Elucidation of the behavior of apudocytes in radiation damage could broaden existing ideas on mechanisms of disturbance of function of the gastrointestinal tract in radiation sickness and could open up new prospects for its restoration.

In the investigation described below postradiation death and restoration of duodenal apudocytes were studied in rats.

EXPERIMENTAL METHOD

An electron-microscopic study was undertaken of the functional morphology of duodenal apudocytes in 45 Wistar rats weighing 200-250 g. The animals were subjected to a single sublethal dose (5 Gy) of whole-body irradiation on the "Luch" gamma-ray source (^{60}Co, dose rate 4.17 Gy/min). Pieces of small intestine taken from the region of the ligament of Treitz after fractional irradiation of the upper half of the rat's abdomen in a total dose of 35 Gy (7 Gy each time, 5 times on alternate days) on the same apparatus (skin-focus distance 45 cm; dose rate 2.14 Gy/min; under these circumstances about 60-65% of the small intestine lay in the zone of irradiation) also were investigated. The animals were killed by decapitation under ether anesthesia 1 h, 1, 3, 7, and 10 days, and 2 and 4 weeks after irradiation. Ultrastructural analysis of the endocrine cells of the small intestine was undertaken on material fixed by Karnovsky's method, under the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

The electron-microscopic study of the rat small intestine showed that 24 h after the end of single-session and fractional irradiation, cytosomes or autophagosomes were seen most frequently in the supranuclear part of the enterocytes in the crypt-bearing part of the small intestine; both whole dying cells at different stages of destruction and cell debris were identified in these structures depending on the time elapsing after their formation (Fig. 1a, c; Fig. 2a, b). The formation of autophagosomes or cytolysomes is not a specific feature of one particular injurious agent. We have observed similar pictures also in animals receiving the cytostatic vincristine [3]. This phenomenon can be regarded rather as the characteristic

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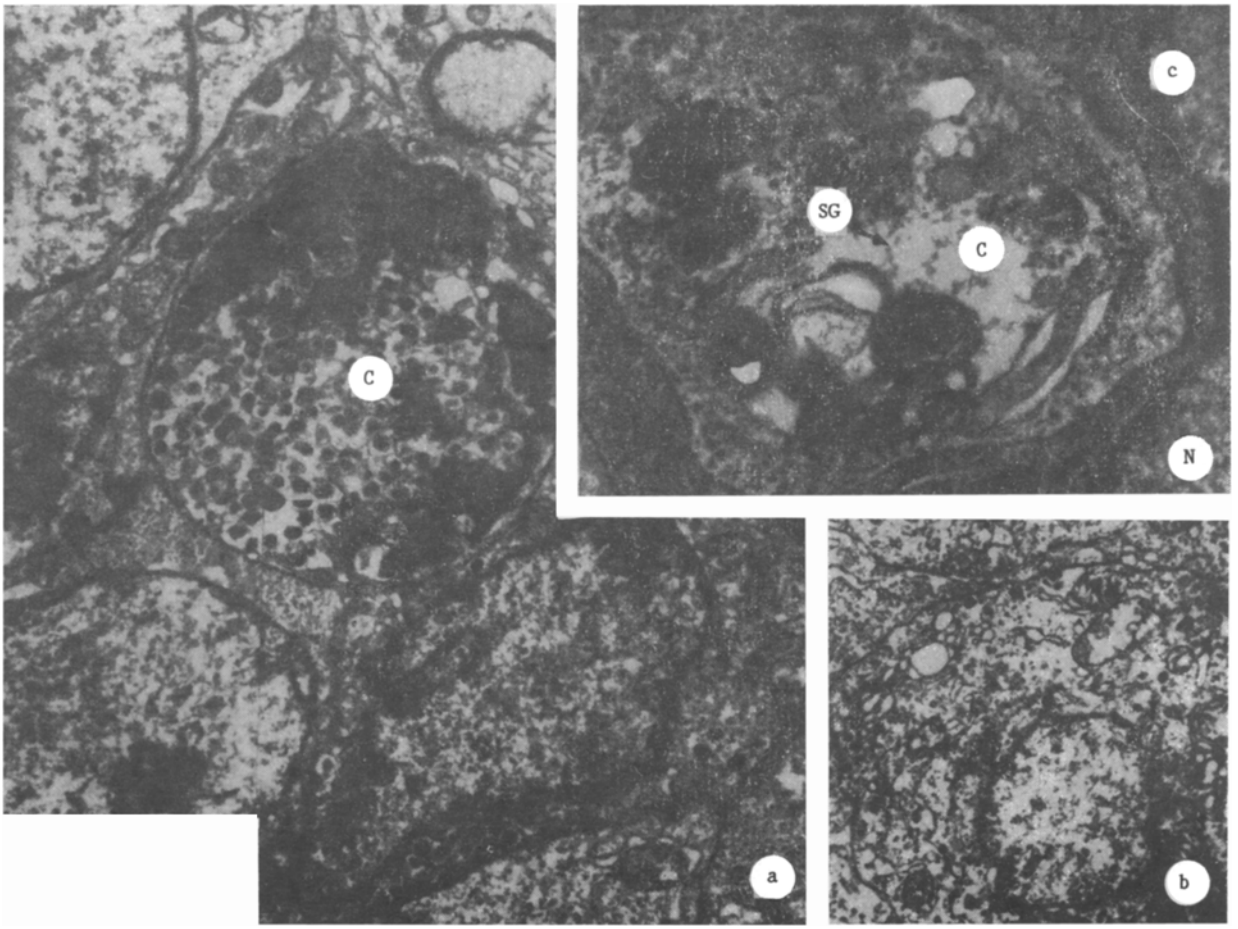

Fig. 1. Rat small intestine after whole-body and fractional irradiation. a) Dying apudocyte in jejunum 10 days after end of course of fractional irradiation of the abdominal region. 7280 ×; b) formation of secretory granules in lamellar complex of enterocytes in crypts of jejunum 8 days after end of fractional irradiation. 7280 ×; c) cytolysome in supranuclear region of enterocyte in duodenal crypt with single secretory granules 24 h after end of whole-body irradiation. 13,650 ×. Here and in Fig. 2: SG) secretory granules, N) nucleus, C) cytolysome, A) autophagosome, V) vacuoles.

response of the enterocytes of the crypt-bearing region of the small intestine to the action of any injurious factor giving rise to destructive changes in the cells. A fact of interest to us is that in some cases it was possible to identify dying apudocytes with clearly distinguishable secretory granules in the contents of the cytolysomes (Fig. 1a, c). Autophagosomes containing debris or sometimes clearly distinguishable secretory granules (Fig. 2a, b) were found sufficiently often directly in the supranuclear zones of the apudocytes.

Among the other ultrastructural responses of the duodenal endocrine cells of the rats to irradiation, vacuolation of the cytoplasm and its degranulation were observed, and were particularly marked 3 days after irradiation. These changes were concerned chiefly with the EC-cells, whereas structural changes at this period of the investigation in apudocytes producing other hormonal products were less marked or were absent altogether. Attention is drawn to a parallel trend in the development of vacuolation and degranulation of the cytoplasm: secretory granules in this case were distributed mainly around the periphery of the vacuoles and in close contact with them (Fig. 2a, b). Often the cytoplasm of the vacuolated cells contained only single secretory granules. This is evidence that degranulation of the EC-cells, like the well known process of exocytosis on the basolateral surfaces of the cells, may take place when the need arises at sufficiently high intensity due to lysis of the secretory product in the vacuoles described above.

EC-cells are known to be among the most widespread types of apudocytes found in organs of the gastrointestinal tract [1, 8]. The hormonal products produced by these cells are

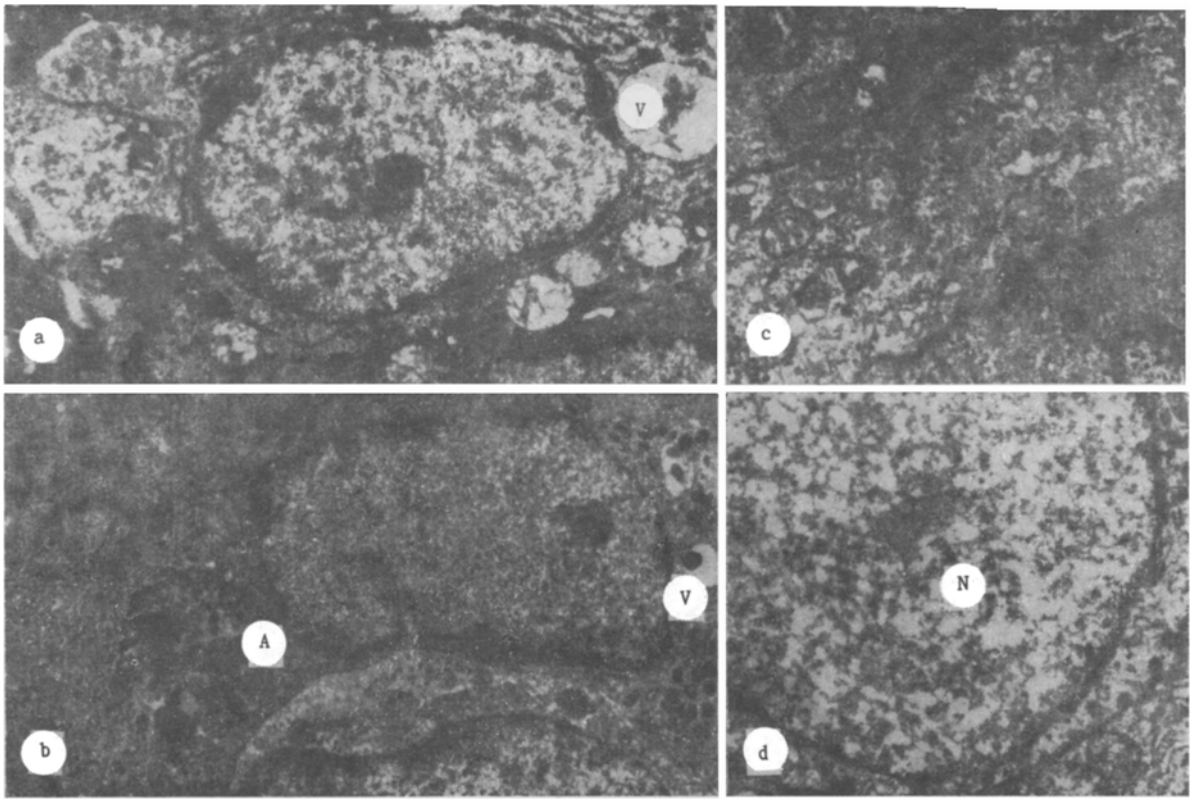

Fig. 2. Rat small intestine after whole-body and fractional irradiation. a) EC-cell in duodenal crypt 3 days after whole-body irradiation, containing autophagosome in supranuclear region. Degranulation and vacuolation of cytoplasm. 7280 ×; b) EC-cell in duodenal crypt, 24 h after whole-body irradiation. Autophagosome in supranuclear region, containing secretory granules. 9100 ×; c, d) formation of secretory granules in enterocytes of jejunal crypts of rats 8 days (c) and 10 days (d) after end of course of fractional irradiation. Magnification: 9100 and 7280 respectively.

dominated by serotonin (up to 90-95% of the total serotonin of the body is produced in them). Serotonin is converted during metabolism into melatonin by methylation of N-acetylserotonin or by acetylation of a second derivative of serotonin, namely 5-methoxytryptamine (mexamine), which possess marked radioprotective properties. The presence of these products in EC-cells has now been firmly established [6, 7].

Taking these data into account, it can be postulated that the ultrastructural response of the EC-cells at different times after irradiation is not accidental but is adaptive in character.

From 8 to 10 days after the end of a course of fractional irradiation of the abdominal region of rats, against the background of activation of repair processes in the mucous membrane of the small intestine, enterocytes whose cytoplasm contained single electron-dense granules of different shapes and sizes were found in the crypts (Fig. 1b; Fig. 2c, d). This can evidently be interpreted as a fact reflecting the possibility of formation of apudocytes, which produce various secretory products, from a stem cell of the intestinal epithelium. The formation of enteroendocrine cells was not observed in the duodenal mucosa of rats subjected to single whole-body irradiation in a sublethal dose.

Postradiation death of small intestinal apudocytes (evidently D_1-cells) after relatively small doses of irradiation has thus been demonstrated for the first time and is evidence of their high radiosensitivity (irreversible injuries and death of A-cells of the islets of Langerhans of the pancreas after superlethal doses of irradiation have been reported in the literature [10]; second, the ability of the enteroendocrine cells of the rat small intestine to form autophagosomes was established. Identification of secretory granules in enterocytes of the crypt-bearing region of the intestinal mucosa during the recovery period after local irradiation of the abdominal region, and also the ability of endocrine cells to form autophagosomes, are objective criteria which support the hypothesis that the epithelial and endocrine cells of the small intestine originate from a single histogenetic anlage (stem cell).

These investigations, revealing differences in the response of different types of apudocytes to ionizing radiation, are evidence of the urgency and necessity of a study of local mechanisms of hormonal homeostasis under extremal influences.

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AUTORADIOGRAPHIC INVESTIGATION OF RNA SYNTHESIS IN KIDNEY TISSUE IN THE EARLY PERIOD OF NECROTIZING NEPHROSIS CAUSED BY MERCURIC CHLORIDE

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Some aspects of the problem of RNA synthesis in kidney tissue in necrotizing nephrosis caused by mercuric chloride have not yet been adequately studied. Considering the importance of such information for the study of the pathogenesis of toxic lesions of the kidneys and for the development of measures aimed at the fastest possible recovery of the damaged organ, it was decided to study, by autoradiography, correlation between the early morphological disturbances and the ability of kidney tissue cells to synthesize RNA.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 150-180 g. The animals were divided into three groups: two rats of group 1 served as the control, animals of groups 2 and 3 (three rats in each group) received mercuric chloride, dissolved in physiological saline, in a dose of 0.5 mg/100 g body weight subcutaneously. The control animals received the equivalent volume of physiological saline. Animals of all groups were given an intraperitoneal injection of ^{3}H-uridine 2 h before sacrifice in a dose of 2 mBq/g body weight (specific radioactivity 370 GBq/liter). Pieces of the kidneys were taken after 24 h from the animals of group 2

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