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ScienceDirect
Regulatory Peptides 136 (2006) 30–39
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Serotonin-producing enterochromaffin (EC) cells of gastrointestinal mucosa in dexamethasone-treated rats

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Received 23 November 2005; received in revised form 19 April 2006; accepted 28 April 2006
Available online 13 June 2006

Abstract

The aim of our study was to investigate the morphological, immunohistochemical and ultrastructural changes of rat serotonin-producing enterochromaffin (EC) cells of gastrointestinal mucosa in dexamethasone-treated rats (D). After 12-daily intraperitoneal administration of 2 mg/kg dexamethasone, rats developed diabetes similar to human diabetes type 2. Stomach, small and large intestines were examined. Large serotonin positive EC cells appeared in the corpus mucosa epithelium of D group of rats, although these cells were not present in control (C) rats. Both volume fraction and the number of EC cells per mm^2 of mucosa were significantly increased only in the duodenum. However, the number of EC cells per circular sections of both antrum and small intestine was increased, but reduced both in the ascending and descending colon in D group. The dexamethasone treatment caused a strong reduction in number of granules in the antral EC cells, while it was gradually increased beginning from the jejunum to descending colon. The mean granular content was reduced in the antral EC cells but increased in the jejunal EC cells in D group. In conclusion, the present study showed that morphological changes in gut serotonin-producing EC cells occurred in diabetic rats.

Keywords: EC cells; Gut; Dexamethasone; Rat

1. Introduction

Intake of food and its processing which occurs inside the gastrointestinal (GI) tract are highly controlled processes. In this control diffuse neuroendocrine system (DNES) has a special importance. It plays an important regulatory role in gastrointestinal motility, secretion, absorption, local immune defense, and cell proliferation [1–3].

Among the members of DNES are EC (enterochromaffin) cells, scattered in the mucosal epithelium of the stomach and throughout the whole length of the intestines [4,5]. These cells synthesize, store and secrete biogenic amine serotonin (5-hydroxytriptamine — 5-HT) [6,7] that is a general stimulator of gut motility and secretion of mucus and digestive enzymes [8]. EC cells release 5-HT in response to an increase of intraluminal pressure or chemical stimuli [9,10] and play a role in the initiation of peristaltic and secretory reflexes [9,11–13].

Serotonin is probably one of the compounds responsible for diarrhea in patients presenting with carcinoid syndrome, suggesting that this amine might modify the intestinal transport process [9,14]. In rodents, it has been shown that nitric oxide is involved in 5-HT-induced fluid secretion [15].

Gastrointestinal disorders have been reported in patients with diabetes mellitus and included esophageal dysmotility, gastroparesis, diarrhea, constipation, fecal incontinence and impaired gall bladder contraction [16–18].

Results of some studies indicate that diabetes condition exerts a significant influence on the GI tract and its DNES in animals in regard to cellular density and neuroendocrine content [19–22]. Gut DNES has been investigated in several animal models of human type 1 diabetes [19,23–25]. There are a few literature data about DNES in animal models of human type 2

Abbreviations: 5-HT; 5-hydroxytriptamine; NIDDM; non-insulin-dependent diabetes mellitus.

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0167-0115/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2006.04.019

diabetes mellitus, and all are concerned about genetic and spontaneous animal models of diabetes mellitus type 2 [20,21].

Among animal models of diabetes mellitus type 2, experimental type 2 diabetes mellitus can be induced by: chemical destruction or surgical removal of part of the B cell mass, lesioning the ventromedial hypothalamus, feeding with high-fat and high-sugar diet, malnutrition in utero, high doses of counter-regulating hormones, particularly glucocorticoids, and prolonged cell exposure to hyperinsulinemia [26].

Since there is no data about the relationship between experimentally induced prediabetic stage or diabetes mellitus type 2 and alteration of gut serotonin-producing cells, the aim of our study was to investigate the morphological, immunohistochemical and ultrastructural changes of rat EC cells of GI mucosa in dexamethasone-treated rats, as a experimental model of prediabetic stage/diabetes mellitus type 2.

2. Materials and methods

2.1. Animals and tissue preparation

Twenty male Wistar rats (Vinča) aged 30 days, and weighting approximately 128 g, were used. Animals were kept in the metabolic cages and fed on a standard diet with water ad libitum. They were kept at a constant temperature and humidity and a light cycle of 12 h on/12 h off. After a few days of acclimatization, rats were randomly allocated into control (C, 10 rats) and dexamethasone-treated (D, 10 rats) animals. The animals were given daily intraperitoneal 2 mg/kg dexamethasone (group D), or saline (group C) for 12 days. Seven rats survived the dexamethasone treatment. After 12 days of experimental period the animals were fasted overnight and the abdomen opened under light ether anesthesia. This protocol was approved by our Animal Use Committee which is similar to the Canadian Council on Animal Care.

2.2. Glucose and insulin determination

Fasting blood and urine glucose levels were measured by the glucose oxidase method [27]. Plasma and pancreatic insulin were measured by radioimmunoassay using commercial kits in accordance with the instructions (INEP-Diagnostics, Zemun) and rat insulin standard (Novo Industry, Bagsvaerd, Denmark).

2.3. Preparation of histological sections

The GI tract from all animals from each group was removed and specimens were obtained from the following parts of the tract: antrum with corpus, duodenum, jejunum, ileum, coecum, ascending and descending colon. Three tissue specimens from each above mentioned part of the GI tract were obtained. The tissue specimens were fixed in Bouin's fluid for 8 h, dehydrated and embedded in paraffin. During the paraffin-embedding process we tried to orientate the specimen with the mucosal surface perpendicular to the cutting surface. Antral and corpus tissues were oriented so that they could be cut perpendicular to the gland's entire length in order to examine carefully the morphology of the mucosa and endocrine cells as well as to avoid quantitative analysis of the same gland. The "ideal" or satisfactory (intact stroma from the base of the mucosa to the top of the villi in the small intestine and crypts in the colon) sections were made. They were cut to 5 μm thickness, and stained by H&E.

2.4. Immunohistochemistry

Immunohistochemistry was performed on 5 μm serial sections from Bouin-fixed paraffin embedded tissue. The monoclonal antiserum against serotonin (DAKO, Denmark, Code No. M0758), working dilution 1:25, used in the present study has been characterized and employed in previous investigations.

2.5. Immunohistochemical protocol

After deparaffinisation in xylene and rehydration through decreasing concentrations of ethanol, slides were immersed in citrate buffer (pH 6.0) (Target Retrieval Solution, ready-to-use; DAKO, S1699) and heated for 21 min in microwave oven at 780 W. After cooling, slides were rinsed in distillated water and treated with 3% H_2O_2 in distillated water for 10 min to reduce endogenous peroxidase activity. Immunostaining was performed by incubating tissue sections with appropriate sera for 60 min at room temperature in humidity chamber, using the streptavidin–biotin technique (LSAB+ Kit, Peroxidase Labeling, K0690, DAKO Cytomation, Denmark). After washing in 0.01 M phosphate buffered saline (PBS, pH 7.4) specimens were incubated with biotinylated anti-mouse, anti-rabbit and anti-goat immunoglobulins for 30 min at room temperature in humidity chamber, and subsequently incubated with peroxidase-conjugated streptavidin–biotin for another 30 min. After incubation, the sections were rinsed in 0.01 M PBS. Antigen–antibody complexes were visualized with 3,3-diamino-benzidine substrate solution (Liquid DAB+ Substrate Chromogen System, Code K3468, DAKO Cytomation, Denmark) or 3-amino-9-ethylcarbazole substrate solution (AEC+ High Sensitivity Substrate Chromogen, Code K3461, DAKO Cytomation, Denmark) and after that washed with distillated water. The cell nuclei were contrastained with Mayer's haematoxylin. The control stainings included omission of the primary antiserum and replacement of the first layer of antibody by non-immune serum diluted 1:10 and by the diluent alone. As a positive control we used samples of normal human colon mucosa which we previously examined by immunohistochemistry.

2.6. Electron microscopy

Tissue samples were taken from five rats from each group. Small blocks were cut and immersed in 3% glutaraldehyde in 0.1 M sodium caccodylate buffer (pH 7.4) for 3 h. All specimens were postfixed for 1 h in 1% osmium-tetraoxide in the same buffer, dehydrated in a series of ethanol concentration and propylene-oxide, and embedded in Epon 812. Ultrathin sections were cut on LKB Ultratome III, double stained with uranyl acetate and lead citrate, and examined under Philips CM 12 electron microscope.