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Changes of Somatostatin-Immunoreactive Cells on the Stomach of Ovariectomized Rats

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The changes on the regional distributions and frequencies of somatostatin-immunoreactive (IR) cells in the fundus and pylorus of the stomach of osteoporotic Sprague-Dawley rats induced by ovariectomy were studied by immunohistochemical methods. The experimental animals were divided into two groups, one for non-ovariectomized group (Sham) and the other for ovariectomized group (OVX). Samples were collected from the fundus and pylorus regions at the 10 th week after ovariectomy or sham-operation. Somatostatin-IR cells were observed in both regions of the stomach regardless of ovariectomy. Most of these IR cells in the mucosa of the fundus or pylorus were generally spherical or spindle in shape (open type cell) while cells found in the gastric gland regions were round in shape (close type cell). Significantly lower number ($P<0.01$) of somatostatin-IR cells were detected in OVX as compared with Sham in the fundus and pylorus. In the present study, the density of somatostatin in the stomach was markedly decreased. Therefore, these changes in density of somatostatin-IR cells detected in this study may support the speculation that the development of gastrointestinal symptoms in osteoporosis such as impairments of calcium and some lipids, frequently encountered in patients with postmenopausal osteoporosis because the changes in gastrointestinal endocrine density would reflect the change in the capacity of producing these hormones and regulating gut motility and digestion.

Key Words: Ovariectomy, Somatostatin-immunoreactive (IR) cells, Stomach

INTRODUCTION

Osteoporosis is caused by an imbalance between bone resorption and bone formation, which results in bone loss and fractures after mineral flux. The frequency of fractures significantly increases in osteoporosis, and hip fractures in senile patients are a very serious problem because it often limits the patients' quality of life. The postmenopausal osteoporosis model using ovariectomized rat is useful for evaluation of osteoporotic drugs, because several parameters clearly decrease by the ovariectomy within 4 weeks after operation (Yamaguchi et al., 1999). In addition, the ovariectomized rat bone loss model is suitable for studying problems that are relevant to postmenopausal bone loss, because ovariectomy that induced bone loss in the rat and postmenopausal bone loss share many similar characteristics including decreased intestinal absorption of calcium (Kalu, 1991).

Gastrointestinal (GI) endocrine cells dispersed in the epithelia and gastric glands of the digestive tract synthesize various kinds of GI hormones and played an important role in the physiological functions of the alimentary tract (Bell, 1979). Until now, the endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and a change in their density would reflect the change in the capacity of producing these hormones (El-Salhy and Sitohy, 2001). Somatostatin consisted of 14 amino acids was isolated from hypothalamus of sheep for the first time and it could be divided into straight form and cyclic form (Brazeau et al., 1973). This substance inhibits the secretion of the other neuroendocrine hormones (Kitamura et al., 1984). It is known that somatostatin-immunoreactive (IR) cells show the widest distribution in the whole GIT except for the large intestine of all vertebrate species inves-

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tigated, including the primitive agnathans with serotonin-IR cells (Ku et al., 2003). Somatostatin-IR cells are significantly increased in the cancer adjacent mucosa compared to that of cancer distant mucosa of colorectal endocrine cancer patients (Zhao et al., 1997) and decrease of these IR cells is demonstrated in the duodenal ulcer patients with *Helicobacter pylori* but they are increased to normal after eradication of *Helicobacter pylori* (Queiroz et al., 1993). Significantly decrease of GI endocrine cells in the ovariectomized osteoporotic rats using silver techniques was previously reported (Ku et al., 2004ab) with marked decreases in chromogranin-IR cells, a marker of endocrine cells in GI tract of ovariectomized rats (Ku et al., 2005). The distribution and frequency of GI endocrine cells are varied with feeding habits (Solcia et al., 1975). Osteoporotic patients and/or animals show quite different feeding habits (Thomas, 2003). However, there is no report dealing the changes of somatoatatin-IR cells at osteoporotic status in spite of some clear disorder of gastric absorption of calcium ion (Kalu and Chen, 1999), and lipids (Loest et al., 2002). Osteoporosis induced by ovariectomy or post-menopause is directly related to some endocrine system especially to estrogen (O'Toole et al., 1985; Riggs, 2002).

The purpose of this study is to demonstrate the changes of regional distribution and frequency of somatostatin-IR cells in the fundus and pylorus regions of the stomach of a postmenopausal osteoporotic rat induced by ovariectomy. In this study, the stomach was sampled at the 10 th week after ovariectomy or sham-operation.

MATERIALS AND METHODS

1. Experimental animals

Twenty Sprague-Dawley female rats (6-wk old upon receipt, Charles River, Japan) were used after acclimatization for 7 days. Animals were allocated 5 per polycarbonate cage in a temperature (20~25℃) and humidity (30~35%) controlled room. Light : dark cycle was 12 hr : 12 hr and feed (Samyang, Korea) and water were supplied free to access. Half of rats were ovariectomized group (OVX) and remainders were sham-operated group (Sham).

2. Bilateral ovariectomy

All rats were anesthetized with Ketamine hydrochloride (60 mg/2 ml/kg) and Xylazine hydrochloride (2.5 mg/2 ml/kg) combination and subjected to operation. Bilateral ovariectomy was performed by removing both ovaries in the abdominal cavity for OVX, and sham-operation (ovary identification) was performed for Sham.

3. Tissue preparation and staining

After phlebotomy, each parts of the fundus and pylorus was collected from all experimental animals at the 10 th week after ovariectomy or sham-operation after 18 hr fasting to GI empty. Collected samples were fixed in Bouin's solution, then embedded in paraffin, sectioned (3~4 μm) and stained with Hematoxylin-Eosin staining for confirming normal architecture of each region of the stomach.

4. Immunohistochemical staining

Each representative section was deparaffinized, rehydrated and immunostained with the peroxidase-anti peroxidase (PAP) method (Sternberger, 1979). Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera against somatostatin (PUO421295, BioGenex Lab., San Ramon, CA, USA; working dilution 1:20). After rinsing in phosphate buffered saline (PBS 0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the IR cells were observed under light microscope. The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (Sternberger, 1979), including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen.

5. Quantity analyses

The frequency of somatostatin-IR cells was calculated using automated image analysis (Soft Image System,

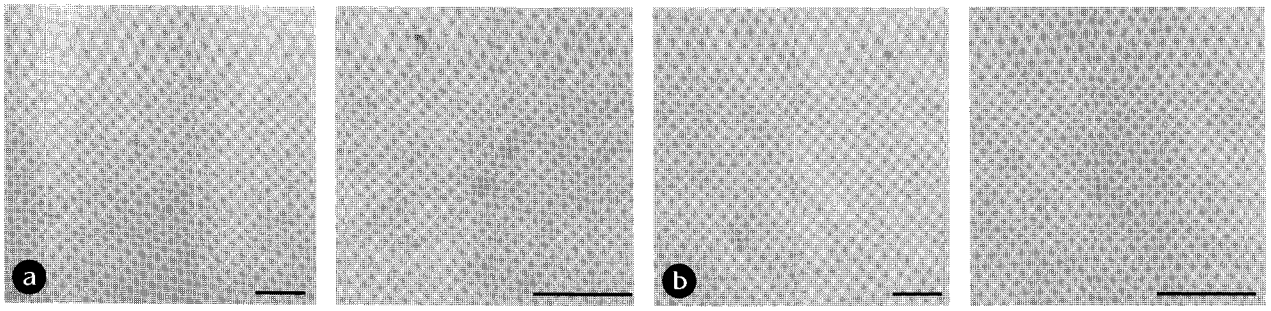

Fig. 1. Somatostatin-IR cells in the fundus of Sham-operated (a) or OVX (b) groups. They were randomly distributed throughout the fundic mucosa mainly basal regions in both groups. However, they were markedly decreased in OVX as compared with Sham. All PAP methods; Scale bars = 100 μm.

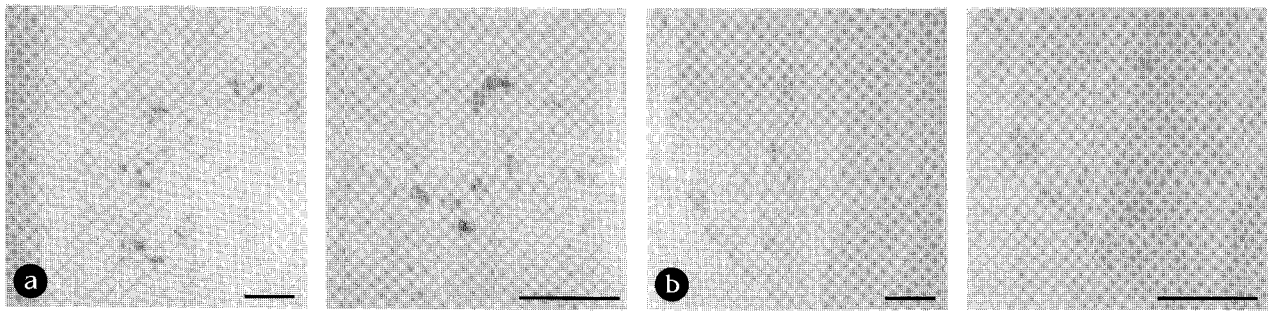

Fig. 2. Distribution of somatostatin-IR cells stained with immunohistochemical staining in the pylorus of Sham-operated (a) or OVX (b) groups. They were randomly distributed throughout the pyloric mucosa mainly basal regions in both groups. However, they were markedly decreased in OVX as compared with Sham. All PAP methods; Scale bars = 100 μm.

Germany) under microscope (Carl Zeiss, Germany) in the uniform area of rectal mucosa among 200 parenchymal cells. IR cell numbers were calculated as cell numbers/200 parenchymal cells.

6. Statistical analysis

Results are expressed as the mean ± standard deviation (n=10). Mann-Whitney U-Wilcoxon Rank Sum W test (M-W test) was used to analyze the significance of data with SPSS for Windows (Release 6.1.3, SPSS, USA) and a *P*-value of less than 0.05 was considered a significant difference. In addition, the percent changes of the number of somatostatin-IR cells in each stomach regions of OVX were calculated in comparision with Sham to help the understanding of the severities of IR cell frequency changes.

RESULTS

In this study, somatostatin-IR cells were detected both in the fundus and pylorus regardless of OVX. Most of them detected in the mucosa of the fundus and pylorus were generally spherical or spindle in shape (open type cell) while cells found in the both pyloric and fundic gastric gland regions were round in shape (close type cell). Somatostatin-IR cells were mainly dispersed in the basal portions of gastric mucosa rather than surface epithelial regions regardless of ovariectomy. However, they were markedly lower in the fundus and pylorus of OVX as compared with Sham (Fig. 1 and 2).

Somatostatin-IR cells in Sham were detected in the fundus and pylorus with 11.60±2.22 and 19.80±2.15 cells/200 parenchymal cells, respectively. In OVX, they were detected as 6.00±1.94 and 9.20±1.23 cells/200 parenchymal cells, respectively. Somatostatin-IR cells in the fundus and pylorus of OVX group were significantly ($P<0.01$) decrease as compared with Sham (Fig. 3). They were decreased as -48.28 and -53.54% in the fundus and pylorus by ovariectomy in the present study, respectively.

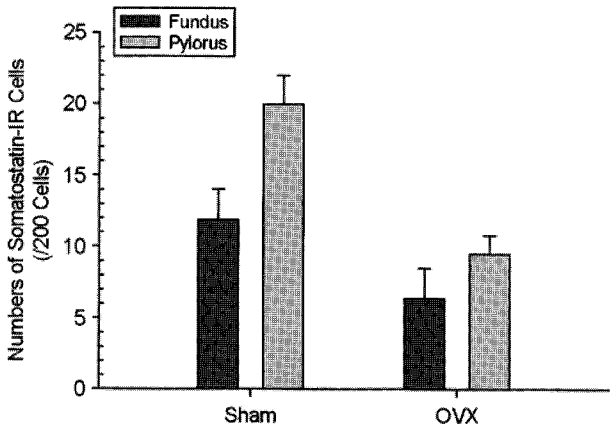

Fig. 3. Number of somatostatin-IR cells in the stomach - fundus and pylorus regions of Sham and OVX groups. Note that somatostatin-IR cells were significantly (*P<0.01) lower in the fundus and pylorus of OVX as compared with Sham. Values are mean ± S.D. of ten rats, /200 cells.

DISCUSSION

It is generally accepted that osteoporosis is metabolic and hormonal disorder that is clearly related to estrogen (O'Toole et al., 1985; Riggs, 2002). The GI endocrine cells are generally divided into two types, one is round to spherical shaped close type cells which are located in the stomach regions, and the other is spherical to spindle shaped open type cells which are situated in the intestinal regions. In addition, the endocrine cells are regarded as the anatomical units responsible for the production of gut hormones, and a change in their density would reflect the change in the capacity of producing these hormones (El-Salhy and Sitohy, 2001). In the present study, the changes of somatostatin-IR cells in the two parts of stomach, the fundus and pylorus of Sprague-Dawley rats after ovariectomy were observed by immunohistochemical technique, the PAP method. Somatostatin-IR cells were significantly (P<0.01) decreased in the both fundus and pylorus regions as results of ovariectomy under same conditions, especially the spindle shaped cells having long cytoplasmic processes. Theses results are well corresponded to that of silver techniques (Ku et al., 2004ab) and that of chromogranins, endocrine markers in ovariectomized osteoporotic rats (Ku et al., 2005). It is generally accepted that the changes of somatostatin-IR cells are clearly related to digestive condition of animals. Until now, somatostatin-IR cells have been variably changed with various disease states (Queiroz et al., 1993; Zhao et al., 1997). It has been postulated that the changes in the GI endocrine cells are a selective process to meet the new demands exerted by the dramatic decrease in intestinal absorption (El-Salhy, 1998) and osteoporotic patients and/or experimental animals shows impairment of absorption of calcium ion (Kalu, and Chen, 1999; Mitamura et al., 2002) and increase of absorption of cholesterol and other lipids (Loest et al., 2002). The decreases of endocrine cells are also detected with aging especially to cells that release the hormone regulating GI motility (Lucini et al., 1999). Therefore, the decrease of the fundic and pyloric somatostatin cells may be responsible for the malabsorption of calcium and lipids that occur in patients with postmenopausal osteoporosis and these decreases of endocrine cells are also detected with aging especially to cells that release the hormone regulating GI motility (Lucini et al., 1999).

In conclusion, ovariectomy induced severe quantitative changes of the gastric somatostatin-IR cell density, and the abnormality in density of GI endocrine cells may contribute to the development of GI symptoms in osteoporosis such as impairments of calcium and some lipids, frequently encountered in patients with postmenopausal osteoporosis. However, changes on other regions of GI tracts, or changes of other endocrine cells are not clear. Further study elucidating the changes of somatostatin-IR cells in other regions of GI tracts or changes on other GI endocrine cells using immunohistochemistry will provide better understanding GI disorder that occurs in various diseases.

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