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Changes of the Somatostatin-immunoreactive Cells in the Pancreas of the Korean Native Goat (*Capra hircus*) during Development

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The distribution and relative frequency of somatostatin-immunoreactive cells in the pancreas were studied during developmental stages (fetus, neonate, 1-month-old, 6-month-old and adult) of the Korean native goat by immunohistochemical methods. Somatostatin-immunoreactive cells were detected in the exocrine of all ages, in the endocrine portions (pancreatic islets) from the neonate, and in the pancreatic duct of the 1-month-old. The relative frequencies of these cells in the pancreatic islets increased with age. However, there were no age-related changes in the relative frequencies of somatostatin-immunoreactive cells in the exocrine and pancreatic duct. Generally, they were distributed in the interacinar spaces, the epithelium of the pancreatic duct, or dispersed in the peripheral zone of the pancreatic islets in all ages. However, clusters consisting of 3-4 cells were also found in the subepithelial connective tissues from the 1-month-old. In addition, the distributions in the endocrine portions of the adult were divided into two patterns: 1) they are dispersed in the marginal regions with moderate or low frequencies, or 2) in the inner zone with high frequencies.

The pancreas of vertebrates consist of two separate parts: the exocrine and endocrine portions (pancreatic islet or islet of Langerhans). The morphological feature and position of the pancreas in vertebrates vary among animals (Dellmann and Brown, 1987). In the Korean native goat, this organ was divided into three parts: the body, left and right lobes (Lee et al., 1995) as the other ruminants (Nickel et al., 1979).

Somatostatin, a tetradecapeptide with molecular weight of 1638, was isolated initially from the hypothalamus. Since the discovery of somatostatin in pituitary extracts, this peptide has been isolated from many other tissues including the pancreas. In the pancreas, somatostatin is synthesized in D cells. The main action of this hormone is to inhibit the release of insulin, glucagon, and gastrointestinal peptides (Hsu and Crump, 1989).

Many studies have elucidated the distribution and relative frequency of somatostatin-immunoreactive cells in the pancreas of various vertebrates (Alumets et al., 1977; Calingasan et al., 1984; Lee and Lee, 1992; Lee et al., 1995). A few studies that deal with the changes in the distribution and relative frequency of these cells according to development stage are available (Alumets et al., 1977; Yoshinari and Daikoku, 1982; Ito et al., 1988). In addition, reports on the change of the distribution and relative frequency of somatostatin-immunoreactive cells in the developing pancreas of the Korean native goat except for the adult stage have been recorded (Lee and Lee, 1992; Lee et al., 1995).

In the present study, the distribution and relative frequency of somatostatin-immunoreactive cells from the pancreas of the Korean native goat were investigated in the fetus, neonate, 1-month-old, 6-month-old and adult stage of development.

Materials and Methods

Each of five healthy fetus (130 d of gestation), neonate, 1-month-old, 6-month-old and adult Korean native goats (*Capra Hircus*) were used without sexual distinction. The animals were anesthetized with Rompun® (Bayer, Korea), and then the canular was inserted into the left common carotid artery to bleed. After phlebotomized, the pancreas was sampled. The sampled pancreas was fixed in Bouins fluid. After paraffin embedding, 3-4 μm serial sections were prepared by routine methods. Each representative sections were deparaffinized, rehydrated

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Table 1. The regional distribution and relative frequency of glucagon-immunoreactive cells in the pancreas of the Korean native goat at various developmental stages

Group	Exocrine	Endocrine	Duct
Fetus	+	-	-
Neonate	+	++	-
One-month-old juvenile	+	++	+
Six-month-old juvenile	+	+++	+
Adult	+	+++	+

+++; numerous, ++; moderate numbers, +; a few, -; not detected.

and immunostained by peroxidase antiperoxidase (PAP) methods (Sternberger, 1979). Blocking of the nonspecific reaction was performed with normal goat serum prior to incubation with the anti-rabbit somatostatin antiserum (BioGenex). After rinsing in phosphate buffered saline (PBS, pH 7.4), the sections were incubated with secondary antiserum. They were then washed in PBS and the PAP complex was prepared. The peroxidase reaction was carried out in a solution (0.02% 3, 3-diaminobenzidine tetrahydrochloride, 0.01% H_2O_2, 0.05 M Tris-HCl buffer, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayers hematoxylin and immunoreactive cells were observed under a light microscope.

Results

Along with the developmental stages, changes in the regional distribution and relative frequencies of somatostatin-immunoreactive cells were observed and summarized in Table 1. Somatostatin-immunoreactive cells were detected in the exocrine of all stages, in the endocrine from the neonate and in the pancreatic duct from the 1-month-old (Table 1). These cells were spherical, round and spindle shaped (Figs. 1-3).

Fetus: Somatostatin-immunoreactive cells were observed in the exocrine portions. A few cells were dispersed in the interacinar space of the exocrine portion. However, no cells were observed in the pancreatic duct and endocrine portions (Fig. 1A).

Neonate: Somatostatin-immunoreactive cells were found in the pancreatic islets and exocrine portions. In the exocrine portions, the cells were singly observed in the interacinar space in low frequency. A Moderate number of immunoreactive cells were dispersed in the marginal zone of the pancreatic islets. However, no cells were observed in the pancreatic duct (Fig. 2A).

1-month-old: Somatostatin-immunoreactive cells were observed in the pancreatic islets, exocrine portions and pancreatic duct. Singly distributed cells were detected in the interacinar space of exocrine portions in low frequency. A Moderate number of cells were found in the peripheral zone of the pancreatic islets. These cells were observed in the epithelium with rare frequency. In

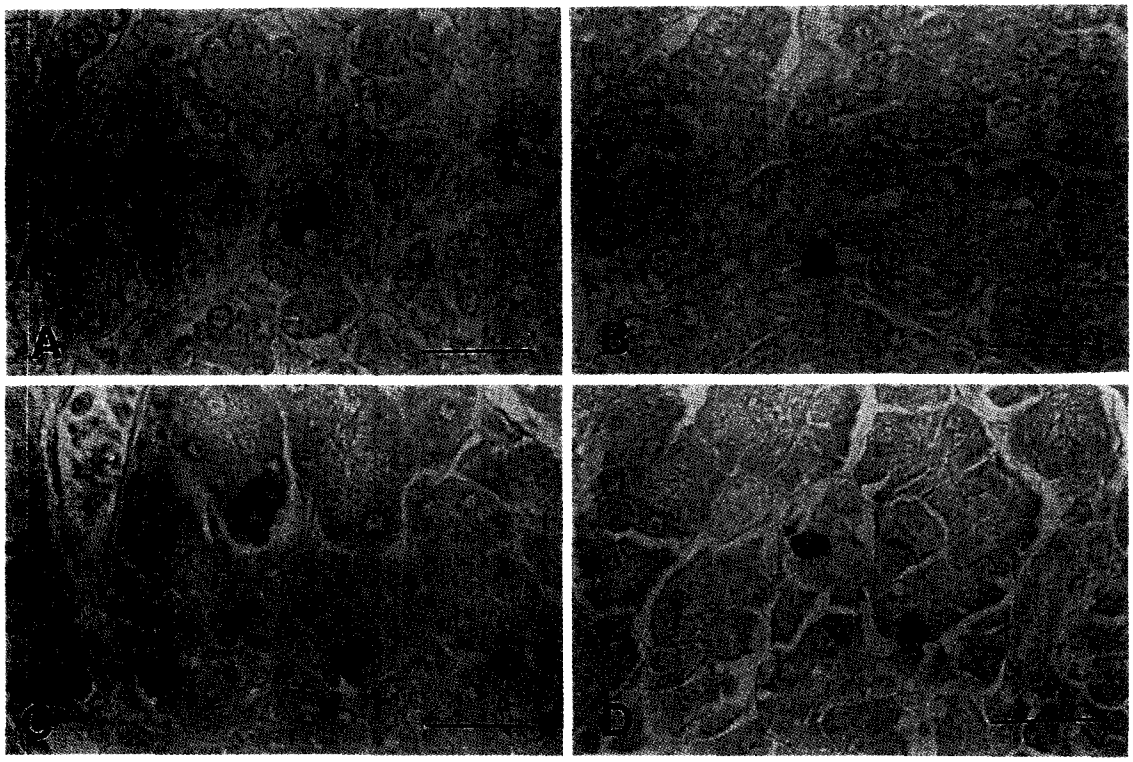

Fig. 1. Somatostatin-immunoreactive cells in the exocrine portions of the pancreas of the Korean native goat. Spherical, round or spindle shaped cells were observed in the interacinus space of the exocrine portions of the fetus (A), neonate (B), six-month-old juvenile (C), and adult (D). Scale bars=50 μm.

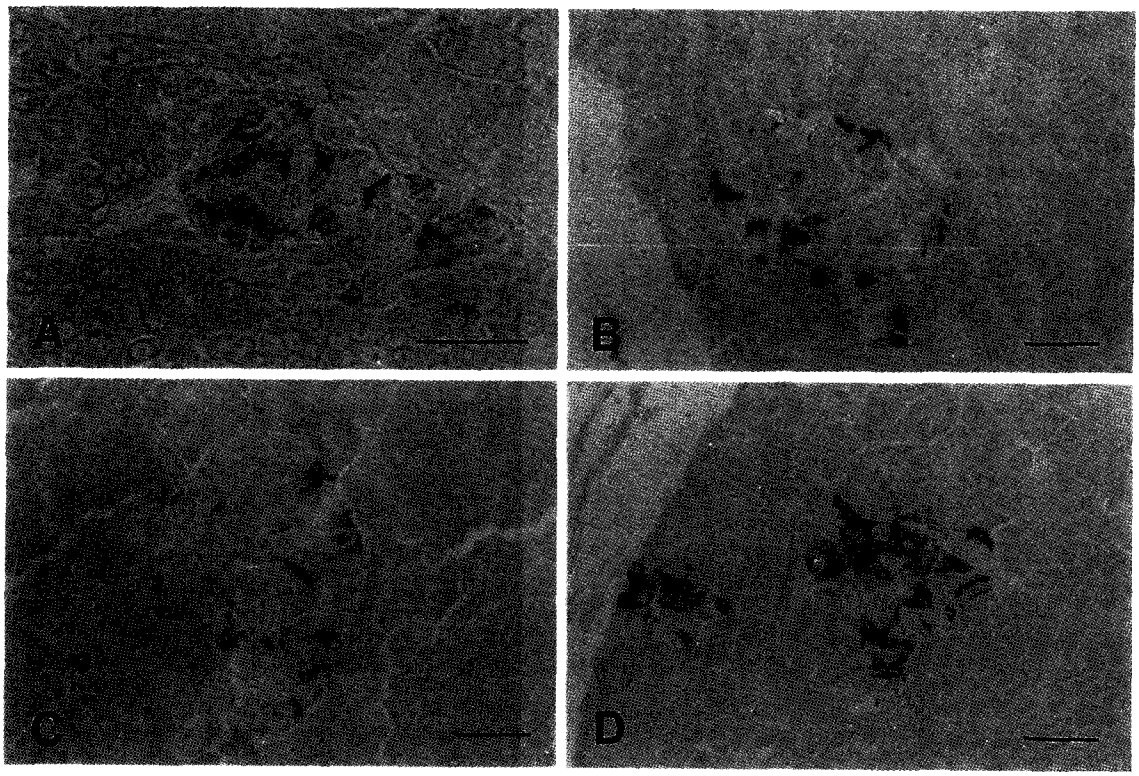

Fig. 2. Somatostatin-immunoreactive cells in the pancreatic islets of the Korean native goat. Spherical, round or spindle shaped cells were observed in the pancreatic islets of the neonate (A), six-month-old juvenile (B) and adult (C, D). Note that the distributions of immunoreactive cells in the pancreatic islets of the adult Korean native goat were divided into two patterns: 1) dispersed in the marginal regions with moderate or low frequencies (C), and 2) in the inner zone with high frequencies (D). Scale bars=50 μm.

addition, clusters consisting of 3-4 cells were detected in the subepithelial connective tissues (Fig. 3A and B). 6-month-old: Somatostatin-immunoreactive cells were detected in the pancreatic islets, exocrine portions and duct. A low frequency of immunoreactive cell was observed in the epithelium of the pancreatic duct and clusters of numerous cells were also detected in the subepithelial connective tissues of the pancreatic duct (Fig. 3C). In the interacinus space of exocrine portions a single or group of 2-3 cells were found with a low frequency (Fig. 1C). Numerous cells were dispersed in the marginal zone of the pancreatic islets (Fig. 2B).

Adult: Somatostatin-immunoreactive cells were detected in the pancreatic islets, exocrine portions and duct, and distribution and relative frequencies of these cells in the exocrine portions and duct were similar to those of the 6-month-old (Fig. 1D, 3D, and E). However, the distributions in the endocrine portions were divided into two patterns: 1) dispersed in the marginal regions with moderate or low frequencies, and 2) in the inner zone with high frequencies (Fig. 2C and D).

Discussion

Somatostatin, which was first isolated from the hypothalamus of sheep, is a polypeptide which consist of 14 amino acids. It exists in two forms: 1) a straight form and 2) a cyclic form. A new type of somatostatin, somatostatin-28, was isolated from the pig (Brazeau et al., 1973). This hormone is identified in the gastrointestinal endocrine system and nervous system. In the gastrointestinal endocrine system, this substance is distributed in the mucosa of the fundus in the stomach and in D cells of the pancreas. The main function of somatostatin is inhibitory action against gastrin, cholecystokinin, secretin, glucagon, gastrin inhibitory peptide, insulin, and motilin, and it inhibits the release of gastric acid and pancreatic fluid (Kitamura et al., 1984). They also inhibit the absorption of amino acid, glucose, and neutral fat in the small intestine. King and Millar (1979) measured the concentrations of somatostatin in animal tissues and reported that the highest concentration of this substance was detected in the pancreas. The distribution and relative frequency of the somatostatin-immunoreactive cells in the pancreas was studied in the human (Erlandson et al., 1976), mink (Kawano et al., 1983), guinea pig (Baskin et al., 1984), frog (Kaung and Elde, 1980), snake (Rhoten, 1984), lungfish (Hansen et al., 1987), *Barbus conchonius* (Rombout et al., 1986), trout (Wagner and Mckeown, 1981), duck (Lucini et al., 1996), mallard (Lee et al., 1998) and other animals (Lee and Lee, 1992). According to previous studies (Erlandson et al., 1976; Kaung

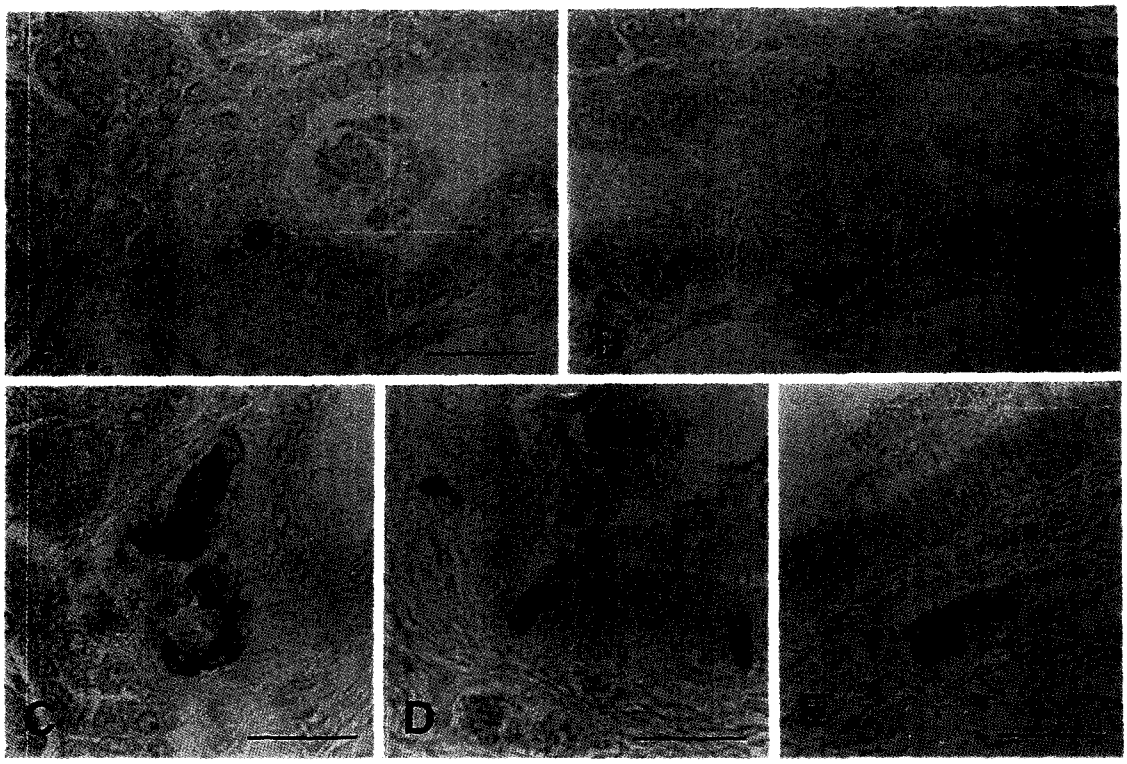

Fig. 3. Somatostatin-immunoreactive cells in the pancreatic duct of the Korean native goat. Spherical, round or spindle shaped cells were observed in the epithelium or subepithelial connective tissue of the 1-month-old juvenile (A, B), 6-month-old juvenile (C) and adult (D, E). Clusters that consisted of moderate to numerous frequency of cells were observed in the subepithelial connective tissue (B, C, E). Scale bars=50 μm.

and Elde, 1980; Wagner and Mckeown, 1981; Kawano et al., 1983; Baskin et al., 1984; Rhoten, 1984; Rombout et al., 1986; Hansen et al., 1987; Lee and Lee, 1992; Lucini et al., 1996; Lee et al., 1998), somatostatin-immunoreactive cells were found in the pancreatic islets, interacinar space of the exocrine portions, and pancreatic duct. However, the distributions of these cells are quite different among the species of animals. In mammals, these cells were generally dispersed in the peripheral zone of the pancreatic islets, but in birds they were dispersed in two regions: 1) the central zone of dark type pancreatic islets, and 2) the peripheral zone of the light or mammalian type pancreatic islets. Hiratsuka et al. (1996) reported that the different distributions of somatostatin-immunoreactive cells were observed in the pancreatic islet of cattle.

The changes in the distribution and the relative frequency of the glucagon-immunoreactive cells in the pancreas by development were reported in the mouse, porcine (Ito et al., 1988), and rat (Alumets et al., 1977; Yoshinari and Daikoku, 1982). According to previous studies (Alumets et al., 1977; Yoshinari and Daikoku, 1982; Ito et al., 1988), the endocrine pancreas continues to differentiate throughout postnatal life. Distributions of somatostatin-immunoreactive cells are quite variable at different development stages. Yoshinari and Daikoku (1982) reported that the appearance of somatostatin-immunoreactive cells were later than other endocrine cells in the pancreas.

In the present study, somatostatin-immunoreactive cells were detected in the exocrine at all developmental stages, in the endocrine portions (pancreatic islets) from the neonate, and in the pancreatic duct from the 1-month-old. Generally, they were distributed in the interacinar spaces, the epithelium of the pancreatic duct or dispersed in the peripheral zone of the pancreatic islets at all ages. These results are similar to those of other mammals (Erlandson et al., 1976; Kawano et al., 1983; Baskin et al., 1984; Lee and Lee, 1992). However, clusters of 3-4 cells were also found in the subepithelial connective tissues from the 1-month-old. In addition, the distributions in the endocrine portions of the adult were divided into two patterns: 1) dispersed in the marginal regions with moderate or low frequencies, and 2) in the inner zone with high frequencies. These changes in the pancreatic islets at various developmental stages are somewhat different from the changes in other mammals (Alumets et al., 1977; Yoshinari and Daikoku, 1982; Ito et al., 1988).

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