

Immunohistochemical study of pancreatic polypeptide-immunoreactive cells in the pancreas of the Korean native goat (*Capra hircus*) during developmental stages

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Abstract : The regional distribution and relative frequency of the pancreatic polypeptide (PP)-immunoreactive cells in the pancreas of the Korean native goat (*Capra hircus*) were studied by immunohistochemical methods (PAP methods) using specific antisera against to PP during developmental stage, fetus, neonate, 1-month-old, 6-month-old and adult.

The different regional distribution and relative frequency of PP-immunoreactive cells in the pancreas of Korean native goat were observed during development. In the exocrine portions, they were detected in the all ages and distributed in the interacinar regions. PP-immunoreactive cells were increased from neonate to 1-month-old but thereafter decreased with developmental stage. In the pancreatic duct, PP-immunoreactive cells were demonstrated from 1-month-old but they were decreased with developmental stages in these regions. These cells were observed in the subepithelial connective tissue of the pancreatic duct with a few frequencies in 1-and 6-month-old. However, they were located in the interepithelial cells of the pancreatic duct in the adult with rare frequency. In the endocrine portion (pancreatic islets), PP-immunoreactive cells were observed from neonate, and the regional distribution of PP-immunoreactive cells from 1-month-old was divided into two patterns : dispersed 1) in the marginal regions of the pancreatic islets with moderate or a few frequencies, and 2) in the whole pancreatic islets (in a case of 1-month-old) or in the central cores (in a case of 6-month-old and adult) with numerous frequency.

In conclusion, the different regional distribution and relative frequency of PP-immunoreactive cells in the pancreas of the Korean native goat were observed during development. It is suggested that the changing of feeding habits and physiological conditions during different developmental stages may cause these differences.

Key words : pancreatic polypeptide, immunohistochemistry, Korean native goat, pancreas, development.

Introduction

The mammalian pancreas was consisted of two separated parts : 1) the exocrine portions and 2) endocrine portions (pancreatic islet or islet of Langerhans). The morphological feature and position of the pancreas was varied among the animals¹. In the Korean native goat, this organ was divided into 3 parts : body, left and right lobes² as the other ruminants³.

Pancreatic polypeptide (PP), 36-amino acid peptide, which is synthesized by F cell in the pancreatic islets, may function as an important feedback inhibitor of pancreatic secretion after a meal⁴. The specific function of this peptide is not clear, however, inhibition of food intake has been postulated as a possible function of this peptide because volume of PP was increased in the bloodstream after meals, especially those in protein⁵. It is suggested that PP stimulates secretion of gastric acid and resolution of glucose in avian liver^{6,7}. Also PP can be used as a marker for endocrine active tumors originating from the pancreas⁸.

Anyway, the changes of the regional distribution and relative frequency of PP-immunoreactive cells or PP producing cells during embryogenesis and neonatal periods have been extensively studied in rodents⁹, mammalia¹⁰, lizard¹¹, amphibia¹², ruminant¹³ and laboratory animals¹⁴. Although, the aspects that these immunoreactive cells were located in the interacinar regions of the exocrine portions and in the marginal portions of the pancreatic islets in mammalian species^{10,13,14} were generally accepted but no reports that dealt with the detail changes of the regional distribution and relative frequency of PP-immunoreactive cells in the developing pancreas of the Korean native goat except for adult stage².

In the present study, the regional distribution and relative frequency of PP-immunoreactive cells in the pancreas of the Korean native goat were investigated in the 5 stages of development : fetus, neonate, 1-month-old, 6-month-old and adult.

Materials and Methods

Each of 5 healthy fetus (130 days 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 RompunTM (Bayer, Korea), and then the canular was inserted into the left common carotid artery to bleed. After phlebotomized, the pancreas was sampled and fixed in Bouin's solution. After paraffin embedding, 3-4 μ m serial sections were prepared with routine histological procedures. Each representative sections were deparaffinized, rehydrated and immunostained with the peroxidase and antiperoxidase (PAP) methods¹⁵. Background blocking was performed with normal goat serum prior to incubation with the anti-rabbit bovine pancreatic polypeptide antiserum (BioGenex Lab, USA). After rinsing in phosphate buffered saline (PBS, 0.01M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3,3-diaminobenzidine tetrahydrochloride containing 0.01% H₂O₂ in Tris-HCl buffer (0.05M, H 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and immunoreactive cells were observed under light microscope.

Results

Among the developmental stages, variable changes of the regional distribution and relative frequency of the PP-immunoreactive cells were observed and summarized in Table 1. PP-immunoreactive cells were detected in the exocrine portions throughout whole developmental stages, in the endocrine portions from neonate and in the pancreatic duct from 1-month-old (Table 1). These cells were spherical, round and/or spindle shaped (Fig 1-3).

Fetus : Clusters consisting of 3-5 PP-immunoreactive cells were restricted to the interacinar regions of the exocrine portions with moderate frequency.

Neonate : PP-immunoreactive cells were observed in the exocrine portions and pancreatic islets. In the exocrine portions, these cells were singly or clustered distributed in the interacinar regions with numerous frequency. Moderate frequency of these immunoreactive cells was dispersed in the

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

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

* Fetus : 130 days of gestation.

Remarks : +++ ; numerous, ++ ; moderated, + ; a few, ; rare, ; not detected.

whole pancreatic islets. However, no cells were observed in the pancreatic duct.

1-month-old : PP-immunoreactive cells were observed in the pancreatic islets, exocrine portions and pancreatic duct. In the exocrine portions, singly or in clusters of two to three cells distributed PP-immunoreactive cells were found in the interacinar regions with numerous frequencies. In endocrine portions, they were separated into two distributional patterns according to the site of appearance with a numerous frequency. One was principally detected in the marginal zone and the other was dispersed in the whole pancreatic islets. Some PP-immunoreactive cells were demonstrated in the subepithelial connective tissue of pancreatic duct with a cluster form consisted of 3-5 cells.

6-month-old : PP-immunoreactive cells were detected in the exocrine portions, endocrine pancreas and pancreatic duct. Moderate frequency of immunoreactive cells were distributed singly or in a cluster of two to three and observed in the interacinar regions of the exocrine portions. In endocrine portions, distribution of PP-immunoreactive cells was separated into two patterns. One was demonstrated in the marginal zone and the other was found in the central cores of the pancreatic islet. In addition, PP-immunoreactive cells were detected in the subepithelial connective tissue of the pancreatic duct with a few frequency as a cluster from.

Adult : PP-immunoreactive cells were detected in the exocrine portions, endocrine pancreas and pancreatic duct. A few frequency of PP-immunoreactive cells were distributed singly or in a cluster of two to three and observed in the in-

teracinar regions of the exocrine portions. In endocrine portions, the distribution of PP-immunoreactive cells was separated into two patterns as 6-month-old according to the site of appearance with numerous frequency. One was detected in the marginal zone and the other was found in the central cores of the pancreatic islet. In addition, these immunoreactive cells were observed among the epithelial cells of the pancreatic duct with rare frequency.

Discussion

Pancreatic polypeptide (PP) was first isolated from polutant mixed in extract of isolation of chick's insulin at 1968, and thereafter have been isolated from cattle, swine and human¹⁶. PP is polypeptides consist of 36 amino acids. Until now, it is generally accepted that PP-immunoreactive cells were distributed in F cells of the pancreas and may function as an important feedback inhibitor of pancreatic secretion after a mea^{14,17}, especially bovine PP inhibited outputs of pancreatic trypsin and bilirubin, without affecting bicarbonate¹⁷ and secretion of gall bladder⁶. But PP increased secretion of gastric acid and glycogenolysis of liver in avian species⁷. And the value of plasma PP as a possible diagnostic marker for pancreatic tumors is as yet unsettled but may be a valuable tool⁴.

The distribution and relative frequency of the PP-immunoreactive cells in the pancreas was studied in the human¹⁸, monkey¹⁹, alligator²⁰, bean goose²¹, duck²², rat⁹, porcine¹⁰, lizard¹¹, *Rana temporaria*¹², *Bubalus bubalis*¹³ and labora-

tory animal¹⁴. According to these previous studies^{9-14,18-22}, it is well known that PP-immunoreactive cells were detected in the pancreatic islet, interacinar regions of the exocrine portion and pancreatic duct. In addition, Ku *et al*²³⁻²⁶ reported that the regional distribution and relative frequency of glucagon-²³, somatostatin-²⁴, chromogranin A-²⁵ and insulin-²⁶ immunoreactive cells in the Korean native goat (*Capra hircus*) were varied with developmental stages and these immunoreactive cells have two distributional patterns, some fixed periods after birth as the PP-immunoreactive cells in this study.

The changes of the regional distribution and relative frequency of PP-immunoreactive cells during embryogenesis and neonatal periods have been extensively studied in rodents⁹, mammalia¹⁰, lizard¹¹, amphibia¹², ruminant¹³ and laboratory animals¹⁴. According to these previous reports⁹⁻¹⁴, the endocrine pancreas continues to differentiate and develop throughout postnatal life and into adulthood, and the distribution of PP-immunoreactive cells are quite different from the developmental stages but there was no reports that demonstrated the different distributional pattern of PP-immunoreactive cells in the pancreatic islets including adult stages.

In addition, the appearance of PP-immunoreactive cells in the pancreas was showed quite different opinion with researchers. Lucini *et al*¹³ suggested that the appearance of PP-immunoreactive cells were later than other endocrine cells in the pancreas but counter researchers to the suggestion of Lucini *et al*¹³, Alumets *et al*²⁷ persisted that these immunoreactive cells were observed from early embryonic periods, 9 days of incubation, in chicken.

Anyway, it is difficult to conclude or persist whether the appearance of PP-immunoreactive cells in the pancreas of the Korean native goat were later than other endocrine cells

because there were no reports about the appearance of these immunoreactive cells in the pancreas of Korean native goat earlier than those of the present study, prior than 130 days of gestation.

In the present study, PP-immunoreactive cells were detected in the exocrine portions of the whole developmental stages, in the endocrine portions of the neonate, 1-month-old, 6-month-old and adult, and in the pancreatic duct of the 1-month-old, 6-month-old, and adult. The relative frequency of these cells was decreased in the exocrine portions and pancreatic duct with developmental stages while increased in the endocrine portions. Generally, they were distributed in the interacinar regions, the epithelium of the pancreatic duct or dispersed in the marginal zone of the pancreatic islets during all developmental stages. These results are similar to those of other mammals^{9,10,13,14}. However, the aspects of clusters of 3-4 cells were also found in the subepithelial connective tissues from the 1-month-old and the distributional patterns in the endocrine portions of the 1-month-old, 6-month-old and adult were divided into two patterns: dispersed 1) in the marginal regions and 2) in the whole pancreatic islets (in a case of 1-month-old) or in central cores of pancreatic islets, was quite different from those of other mammals^{9,10,13,14}. And it was considered that these differences result from the differences of animal species and sampling portions according to reports of Alumets *et al*²⁷ and Gabe²⁸.

In conclusion, the different regional distribution and relative frequency of PP-immunoreactive cells in the pancreas of the Korean native goat were observed during development. It is suggested that the changing of feeding habits and physiological conditions during different developmental stages may cause these differences.

Legends for figures

Fig 1. PP-immunoreactive cells in the exocrine portions of the Korean native goat during developmental stages.

a. fetus b, c. neonate d. 1-month-old
e. 6-months-old f. adult
a, b : 240; c-f : 480, PAP methods

Fig 2. PP-immunoreactive cells in the pancreatic duct regions of Korean native goat during developmental stages.

a. 1-month-old b. 6-months-old c. adult
a : 240; b, c : 480, PAP methods

Fig 3. PP-immunoreactive cells in the pancreatic islets of the Korean native goat during developmental stages.

a. neonate b, c. 1-month-old d, e. 6-month-old
f, g. adult
a-g : 120, PAP methods ; Note that two different distributional patterns were observed in the pancreatic islets from 1-month-old.

References

1. Dellmann H, Brown EM. Textbook of veterinary histology. 3rd ed, Saunders Company, Philadelphia, 1987.
2. Lee HS, Lee IS, Kang TC, *et al*. Immunohistochemical study on the insulin-, glucagon-, somatostatin- and pancreatic polypeptide-secreting cells in Korean native goat. *Korean J Vet Res*, 35:45-54, 1995.
3. Nickel R, Schummer A, Seiferle E. The viscera of the domestic mammals. 2nd ed. Berlin : Verlag Paul Parey. 1979.
4. Lonovics J, Devitt P, Watson LC, *et al*. Pancreatic polypeptide. A review. *Arch Surg*, 116:1256-1264, 1981.
5. Hsu WH, Crump MH. The endocrine pancreas. In McDonald LE, Pineda MH eds, Veterinary endocrinology and reproduction. Lea & Febiger, Philadelphia, pp. 186-201, 1989.
6. Polak JM, Adrian TE, Bryant MG, *et al*. Pancreatic polypeptide in the insulinomas, gastrinomas and glucagonomas. *Lancet*, 1:328-330, 1976.
7. Yamada J, Kitamura N, Yamashita T. Avian endocrinology. In Avian gastrointestinal endocrine cells. Ed, Mikami S, Tokyo : *Japan Sci Soc Press*, pp. 67-69, 1983.
8. Wirth HP, Eberle C, Gautschi K, *et al*. Abnormal increase in pancreatic polypeptide in the secretin-provocation test : hypoglycemia-induced? *Schweiz Med Wochenschr*, 125:735-739, 1995.
9. Fujii S. Development of pancreatic endocrine cells in the rat fetus. *Arch Histol Jpn*, 42:467-479, 1979.
10. Alumets J, H kanson R, Sundler F. Ontogeny of endocrine cells in porcine gut and pancreas. An immunocytochemical study. *Gastroenterology*, 85:1359-1372, 1983.
11. Rhoten WB, Hall CE. An immunocytochemical study of the cytogenesis of pancreatic endocrine cells in the lizard, *Anolis carolinensis*. *Am J Anat*, 163:181-193, 1982.
12. Ortiz de Zarate A, Villaro AC, Etayo JC, *et al*. Development of the endocrine pancreas during larval phases of *Rana temporaria*. An immunocytochemical and ultrastructural study. *Cell Tissue Res*, 264:139-150, 1991.
13. Lucini C, Castaldo L, Lai O, *et al*. Ontogeny, post-natal development and ageing of endocrine pancreas in *Bubalus bubalis*. *J Anat*, 192:417-424, 1998.
14. Wiczorek G, Pospischil A, Perentes EA. Comparative immunohistochemical study of pancreatic islets in laboratory animals (rats, dogs, minipigs, nonhuman primates). *Exp Toxicol Pathol*, 50:151-172, 1998.
15. Sternberger LA. The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In Immunocytochemistry, Sternberger LA, ed, John Wiley & Sons, New York, pp. 104-169, 1979.
16. Lee MS. Immunohistochemical study of the endocrine cell in the gastrointestinal tract of the Korean native goat. Thesis of Ph D in Catholic University of Taegu-Hyosung, pp. 1-94, 1996
17. Greengerg GR, McCloy RF, Chadwick VS, *et al*. Effect of bovine pancreatic polypeptide on basal pancreatic and biliary outputs in man. *Dig Dis Sci*, 24:11-14, 1979.
18. Hahn von Dorsche H, Falt K, Titlbach M, *et al*. Immunohistochemical, morphometric and ultrastructural investigations of the early development of insulin, somatostatin, glucagon and PP cells in fetal human pancreas. *Diabetes Res*, 12:51-56, 1989.
19. Wolfe Coote S, Louw J, Woodroof C, *et al*. Development, differentiation and regeneration potential of the velvet monkey endocrine pancreas. *Microsc Res Tech*, 43:322-331, 1998.
20. Jackintell LA, Lance VA. Ontogeny and regional distribution of hormone-producing cells in the embryonic pancreas of *Alligator mississippiensis*. *Gen Com Endocrinol*, 94:244-266, 1994.
21. Lee JH, Ku SK, Lee HS. An immunohistochemical study of the endocrine pancreas of the bean goose, *Anser fabalis*, Latham. *Korean J Vet Res*, 39:448-454, 1999.
22. Lee JH, Ku SK, Lee HS. Immunohistochemical study on the bovine chromogranin, serotonin and bovine pancreatic polypeptide immunoreactive cells in the pancreas

- of the duck (*Anas platyrhynchos platyrhynchos*, Linne). *Korean J Vet Res*, 38:232-238, 1998.
23. Ku SK, Lee HS, Lee JH. Immunohistochemistry of glucagon-immunoreactive cells in the developing pancreas of Korean native goat (*Capra hircus*). *Korean J Biol Sci*, 3:187-191, 1999.
24. Ku SK, Lee HS, Lee JH. Changes of the somatostatin-immunoreactive cells in the pancreas of Korean native goat (*Capra hircus*) with developmental stages. *Korean J Lab Anim Sci*, 15:195-199, 1999.
25. Ku SK, Lee HS, Park KD, et al. An immunohistochemical study on the chromogranin A-immunoreactive cells in the developing pancreas of Korean native goat (*Capra hircus*). *Korean J Biol Sci*, 3:187-191, 1999.
26. Ku SK, Lee HS, Lee JH. Immunohistochemical study on the insulin-immunoreactive cells in the developing pancreas of the Korean native goat (*Capra hircus*). *Korean J Vet Res*, 39:673-678, 1999.
27. Alumets J, H kanson R, Sundler F. Distribution, ontogeny, and ultrastructure of pancreatic polypeptide (PP) cells in the pancreas and gut of the chicken. *Cell Tissue Res*, 194:377-386, 1978.
28. Gabe M. Donn es histologiques sur les cellules endocrines gastroduodénales des amphibiens. *Arch Histol Jap*, 35:51-81, 1972.
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발생단계에 따른 한국재래산양 췌장에서 pancreatic polypeptide 면역반응세포에 대한 면역조직화학적 연구

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국문초록 : 태아기, 신생아기, 생후 1개월령, 6개월령 및 성체의 한국재래산양 췌장에서 pancreatic polypeptide(PP) 면역반응세포의 분포 및 출현빈도를 PAP 법에 의해 검색하였다.

외분비부에서는 모든 연령에서 관찰되었으며 췌장세포 사이에 분포하였다. PP 면역반응세포는 신생아기에서 생후 1개월에 증가한 후 점차 감소하였다. 췌도관에서는 PP 면역반응세포가 생후 1개월부터 관찰되며 그후 점차 감소하였다. 이들 면역반응세포는 생후 1개월령 및 6개월령의 췌도관 상피하 결합조직에서 소수 관찰되었다. 그러나 성체에서는 췌도관 상피세포 사이에서 극소수 관찰되었다. 내분비부(췌장섬)에서 PP 면역반응세포는 신생아기부터 관찰되며, 생후 1개월령부터의 PP 면역반응세포의 분포는 췌장성 주변부에 중등도 또는 소수가 산재하는 것과 다수의 PP 면역반응세포가 췌도 전체(생후 1개월령) 또는 췌도 중앙부(생후 6개월령)에 산재하는 것 등 두가지 유형을 나타내었다.

결론적으로 한국 재래산양의 췌장에 있어서 PP 면역반응세포의 분포와 출현빈도는 발생단계에 따라 상이하였다. 이는 발생단계 동안의 식이성 및 생리학적 조건변화가 이와같은 상이함을 유발하는 요인이 된 것으로 사료된다.

Key words : pancreatic polypeptide, immunohistochemistry, Korean native goat, pancreas, development.