大韓獸醫學會誌(1993) 第33券 第4號
Korean Vet Res (1993) 33(4) : 573~577

Immunohistochemical studies of glucagon and somatostatin in the pancreas of the Korean tree squirrel, *Sciurus vulgar is corea*

Hyeung-sik Lee, Jae-hyun Lee*
Department of Biology, Kyungsan University
*College of Veterinary Medicine, Kyungpook National University**
(Received september 3, 1993)

청설모췌장의 glucageon과 somatostatin 세포의 면역조직학적 연구

이 형 식·이 재 현*
경산대학교 생물학과
경북대학교 수의학과대학*
(1993년 9월 3일 접수)

초록 : 청설모(*Sciurus vulgaris corea*)의 췌장에 분포하는 glucagon 및 somatostatin 세포의 분포와 그들 분비과립의 특징을 밝히기 위해 면역조직화학적 및 면역전자현미경적 방법으로 관찰하였다. Glucagon 면역반응세포는 주로 췌도의 주변부에 분포하였고, 때로는 외분비부에서 관찰되었다. 이 세포의 분비과립은 직경이 240~320nm였으며, 전자밀도가 높은 core를 전자밀도가 낮은 halo가 둘러싸고 특히 gold particle들은 전자밀도가 높은 core에 강한 반응을 보였다. Somatostatin면역반응세포는 췌도의 주변부를 따를 면역반응이 아주 약한 세포들로 산재하였으며 또한 외분비에서 단독 혹은 소집단으로 관찰되었다. 이 세포의 분비과립은 전자밀도가 낮고 직경이 250~275nm였으며, gold particle는 분비과립에 중등도로 고른 반응을 보였다.

Key words : Korean tree squirrel, glucagon, somatostatin, pancreas, protein A-gold technique.

Introduction

The pancreatic endocrine cells of the vertebrates are composed the four types, namely insulin-, glucagon-, somatostatin- and pancreatic polypeptide (pp)-immunoreactive cells. Recently, improvements in the methods of peptide isolation led to the localisation of motilin-[1], glicentin-[2~4], 5-HT-[5,6] and chromogranin-immunoreactive cells.[7]

Although pancreatic peptides have been investigated on the various vertebrates, the differences in distribution and proportions of these peptides habe been diversified considerably according to species.[8]

Immunohistochemical works of the Korean tree squirrels have only been done on the gastrointestinal tract[9] and pancreatic peptides, insulin-, BPP- and 5-HT-immunoreactive cells.[10]

The purpose of the present work was designed to clarify the distribution and the morphology of the secretory granules to glucagon- and somatostatin-immunoreactive cells in the pancreas of the Korean tree squirrels, *Sciurus vulgaris corea*, using the PAP and protein A-gold technique, respectively.

Materials and Methods

The pancreas from five adult Korean tree squirrels, *Sciurus vulgaris corea*, were used in this study.

Immunohistochemistry : Samples of the pancreas were fixed in Bouin's fluid. After dehydration in alcohol series followed by paraffin embedding 4 μm histological sections were prepared. The representative sections were then deparaffinized, rehydrated and immunostained with the peroxidase-antiperoxidse (PAP) method.[11] Specific antisera used in this study wer anti-glucagon(Immunonuclear Corp., Stillwater, Lot. 8635013, 1:800) and anti-somatostatin (Cambride Research Biochemical Billerica, Lot. C-A325, 1:1,000). The antigen-antibody reaction was visualized by 3, 3'-diaminobenzidin-H_2O_2 solution. After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin.

Protein A-gold immunoelectron microscopic techniqu[12] : The tissues of pancrease were fixed in 0.5% glutaraldehyde and 4% para-formaldehyde mixture buffered with 0.1M cacodylate sodium(pH 7.4) for 4hr at 4℃. The tissues were embedded in Lowicryl K4M(Chemische Werke Lowi, Germany), polymerized at −35℃ for 24hr and finally at room temperature for 3 to 4 days. The ultrathin sections were incubated with above the same antisera, and then with colloidal gole(15nm)-labelled protein A complex(E-Y Lab., Inc., U.S.A.) at room temperture for 30 to 60 min. They were stained with aqueous uranyl acetate and lead citrate, and examined with a HITACHI H-U-12 electron microscope at 75kV.

Results

Light microscopy : In the endocrine portions, glucagon-immuno-reactive cells were usually situated in the periphery and occasionally in the central region of the pancreatic islets(Figs 1a, b, d). Also, isolated immunoreactive cell was found between the pancreatic acinar cells(Fig 1c). Most of these cells were appeared polygonal having cytoplasmic processes that extend near or between adjacent cells(Fig 1d).

Although somatostatin-immunoreactive cells appeared oval shaped were seen in scattered along the peripheral pancreatic islets, they were very weakly reacted in staining(Fig 2a). But occassionally these cells were located as singly or small groups within the pancreatic acinar cells(Figs 2a, b).

Immunoelectron microscopy : Glucagon and somatostatin could be identified in the pancreatic islets of the Korean tree squirrel by the protein A-gold technique.

Glucagon cells contained the large secretory granules which were round or oval shaped with a diameter of 240 to 320nm. Between the electron dense core and the limting membrane of the granule, there was an moderately electron lucent, namely halo of variable width. Although gold particles were observed on a entire granule, the core of the granule was labelled strongly with gold particles-(Fig 1e).

Somatostatin cell displayed the large secretory granules which were spherical with a diameter of 250 to 275nm. The granules were moderately electron opaque and had a very closely attached membrane with no halo. On the other hand, gold particles were mostly demonstrated over the lucent core of the entire granule(Fig 2c).

Discussion

In the pancreatic islets, the typical peripheral distributions of glucagon-, somatostatin-, glicentin- and BPP- immunoreactive cells and the central location of insulin- imraunoreactive cells are well known in various vertebrates.

In the Korean tree squrirrel, Lee[10] reported that insulin-immunoreactive cells were distributed throughout the islets and also as single cell or as small group, on the other hand BPP- and 5-HT-immunoreactive cells were devoid of the islet but scattered the pancreatic acinar cells and the epithelium of the secretory duct, respectively.

In the present study, although the distributions of glucagon- and somatostatin-immunoreactive cells were similar to those of other mammals[16~18,21], somatostatin-immunoreactive cells were weakly stained in the pancreatic islets. Maybe this finding is due to our failure to detect the immunohistological reactivity stemming from species differences at the molecular level, still remains to be clarified.

Ultrastructurally, four cell types were found to differ from one another by the appearances of their secretory granules.[13]

Rectnt developments in immunoelectron microscopic method have contributed to identify the localisation of the specific granules in various endocrine cells. In paticular, the localisation of individual regulatory petpides to a specific ell was achieved by labbeling gold particles that indicated the presence of hormones localised nearly exclusively over the secretory granules of the endocrine

cells.[14]

In this study, the secretory granules labelled strongly with gold particles of glucagon cells displayed a round or oval with a diamether 240 to 320nm, and electron dense core usually surrounded by a halo of less dense granular material. The morphology of secretory granules appeared a similar pattern to that reported earlier.[15~17]

Immunocytochemically, gold particles were demonstrated in localisation of glucagon by Pelletier[15,18,19] and Leigh and Edwin.[17] In man and the Australian fat-tailed dunnart, immunostaining was mostly detected over the dense core of the glucagon of the secretory granules.[15] This pattern of indicating the presence of glucagon is similar to that the Korean tree squirrel.

Immunocytochemically, numerous gold particles were mostly demonstrated in the secretory granules in localisation of somatostatin cells. These cells were smaller than the sceretory granules of other animals, moderately electron lucent and no halo were similar to those of others.[18,20,21] Immunostaining for somatostatin was mostly detected in the secretory granules.

This present study on the Korean tree squirrel represents the distributions, as well as the morphology of the specific granules in glucagon- and somatostatin-immunoreactive cells using the various immunohistochemical techniques.

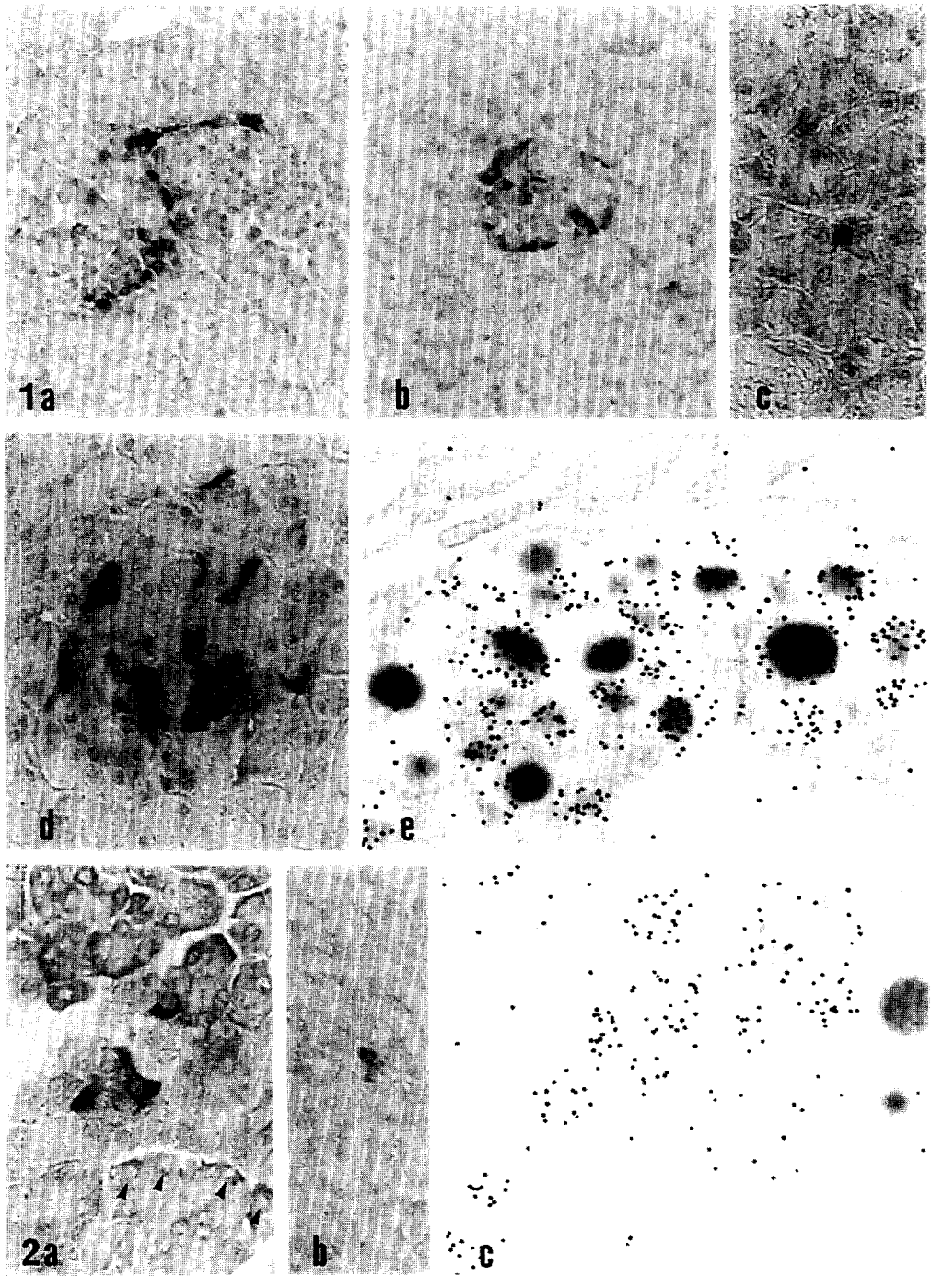
Summary

The pancreatic endocrine cells, glucagon and somatostatin, of the Korean tree squirrel. *Sciurus vulgais corea*, were investigated by means of light and electron microscopic immunohistochemistry using the PAP and protein A-gold techniques. Glucagon-immunoreactive cells were distr.buted the periphery and occasinonaly central region of the pancreatic islets. Also, isolated cell was found between the pancreatic ancinar cells. The glucagon cells contraine granules with a diameter of 240~320nm and the electron dense core usually surrounded by a halo of less dense granular material. The core of granule was labelled strongly with gold particles. Somatostatin-immunoreactive cells were weakly stained in scattered along the peripheral pancreatic islets and were distributed as singly or small groups with in the pancreatic acinar cells. The somatostatin cells were spherical with a diameter of 250~275nm, moderately electron opaque. Gold particles were mostly demonstrated on the entire granule.

Legends of figures

Fig 1a-e. Glucagon-immunoreactive cells were distribution of the periphery of the pancreatic islets(a, b, d), and scattered as sinfle cell between the pancreatic ancinar cells(c). Numerous gold particles indicating the presence of glucagon were found in dense core secretory granules surround by a clear halo. Lowicryl K4M resin(e).
a, b : × 240, c, d : × 540, e : × 50,000

Fig 2a-c. Somatostatin-immunoreactive cells were weakly stained in scattered along the peripheral pancreatic islets(arrow-heads), also observed as single cell or as small groups between the pancreatic acinar cells(a, b). Moderate gold particles indicating the presence of somatostatin were demonstrated in lucent dense secretory granules. Lowicryl K4M resin(c).
a : × 540, b : × 240, c : × 40,000


1a
b
c
d
e
2a
b
c

References

1. Yamada J, Campos VJM, Kitamura N. An immunohistochemical study of endocrine cells in the pancreas of *Caiman katirostris*(Alligatorinae), with special reference to pancreatic motilin cells. *Biomedical lesearch* 1986 ; 7 : 199~208.
2. Calingasan NY, Kitamura N, Yamada J, et al. Immunocytochemical study of the gastro-entero-pancreatic endocrine cells of the sheep. *Acta Anat* 1984 ; 188 : 171~180.
3. Ohara N, Kitamura N, Yamada J, et al. Immunohistochemical study of gastroenteropancreatic endocrine cells of the herbivorous Japanese field vole, *Microtus montebelli*. *Res Vet Sci* 1986 ; 41 : 21~27.
4. Kawano H, Yamashita T, Yamada J, et al. A light microscopic study of the gastro-entero-pancreatic endocrine cells of the mink(*Mustela vison*). *Arch Histol Jap* 1983 ; 46 : 559~573.
5. Nakajima S, Kitamura N, Yamada J, et al. Immunohistochemical study on the endocrine pancreas of cattle with special reference to coexistence of serotonin and glucagon or bovine pancreatic polypeptide. *Acta Anat* 1988 ; 131 : 235~240.
6. Krause WJ, Cuttis Ⅲ JH, Cuttis JH, et al. Immunohistochemical study of the developing endocrine pancreas of the opossum (*Didelphis virginiana*). *Acta Anat* 1989 ; 135 : 84~96.
7. Ito H, Hashimoto Y, Kitamura H, et al. Distribution of Chromogranin containing cells in the porcine gastro-entero-pancreatic endocrine system. *Jap J Vet Sci* 1987 ; 50 : 395~404.
8. Lee JH, Lee HS. Immunohistochemical studies of the pancreatic endocrine cells of the various animals. *Korean J Vet Res* 1992 ; 32 : 497~510.
9. Lee HS, Hashimoto Y, Kon Y, et al. An immunohistochemical study of the gastro-entero-pancreatic endocrine cells in the alimentary tract of the Korean tree squirrel, *Sciurus vulgaris corea*. *Jap J Vet Res* 1991 ; 39 : 117~131.
10. Lee HS. Immunohistochemical study of insulin, BPP and 5-HT peptides in the pancreas of the Korean tree squirrel, *Scirus vulgaris cores*. *J Kyungsan Univ* 1992 ; 10 : 347~355.
11. Sternberger LA. Immunohistochemistry, 2nd ed. 104~169, New York : John Wiley & Sons.
12. Roth J. The protein A-gold technique for antigen localisation in tissue sections by light and electron microscopy. *In* : Immunolabelling for electron microscopy, 113~121, eds. Polak JM & Varndell IM. New York : Elsevier.
13. Lacy PE, Greider MH. Anatomy and ultrastructural organisation of pancreatic islets. In : Endocrinology, 907~919, ed. Groot LJ. New York : Grune and Stratton.
14. Rhoten WB. Immunocytochemical localization of four hormones in the pancreas of the garter snake. *Anat Rec* 1984 ; 208 : 133~242.
15. Pelletier G. Cell types of endocrine pancreas by immunoelectron microscopy. In : Ultrastructual endocrine cells and tissues, 136~140, ed. Motta PM. Boston : Martinus Nijhost Publishers.
16. Edwin N, Leight CM. Immunocytochemical study of the endocrine pancereas in the gray kangaroo (*Macropus fuliginosus*). *Cell Tissue Res* 1990 ; 259 : 183~185.
17. Leight CM, Edwin N. An Immunocytochemical study of the endocrine pancreas in the Australian fat-tailed dunnart (*Sminthopsis crassicaudata*). *Cell Tissue Res* 1991 ; 263 : 195~198.
18. Pelletier G. Identification of four cell types in the human endocrine pancreas by immunoelectron microscopy. *Diabetes* 1977 ; 26 : 749~756.
19. Pelletier G. Immunohistochemical localization of somatostatin. In : Progress in Histochemistry and Cytochemistry vol. 12, 1~40, ed. Fischer G. Springer, Stuttgart, New York.
20. Buchan AMJ, Lance V, Polak JM. The endocrine panceras of *Alligator mississippiensis*. An immunocytochemical investigation *Cell Tissue Res* 1982 ; 224 : 117~128.
21. Forssman A. The ultrastructure of the cell types in the endocrine pancreas of the Horse. *Cell Tissue Res* 1976 ; 167 : 179~195.